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(54) Title: ENVIRONMENTAL STRESS TOLERANCE GENES

(57) Abstract: Recombinant polynucleotides and methods for modifying the phenotype of a plant are provided. In particular, the phenotype that is being modified is a plant's environmental stress tolerance.

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ENVIRONMENTAL STRESS TOLERANCE GENES

RELATED APPLICATION INFORMATION

The present invention claims the benefit from US Provisional Patent Application Serial Nos. 60/166,228 filed November 17, 1999 and 60/197,899 filed April 17, 2000 and "Plant Trait Modification III" filed August 22, 2000.

FIELD OF THE INVENTION

This invention relates to the field of plant biology. More particularly, the present invention pertains to compositions and methods for phenotypically modifying a plant.

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BACKGROUND OF THE INVENTION

Transcription factors can modulate gene expression, either increasing or decreasing (inducing or repressing) the rate of transcription. This modulation results in differential levels of gene expression at various developmental stages, in different tissues and cell types, and in response to different exogenous (e.g., environmental) and endogenous stimuli throughout the life cycle of the organism.

Because transcription factors are key controlling elements of biological pathways, altering the expression levels of one or more transcription factors can change entire biological pathways in an organism. For example, manipulation of the levels of selected transcription factors may result in increased expression of economically useful proteins or metabolic chemicals in plants or to improve other agriculturally relevant characteristics. Conversely, blocked or reduced expression of a transcription factor may reduce biosynthesis of unwanted compounds or remove an undesirable trait. Therefore, manipulating transcription factor levels in a plant offers tremendous potential in agricultural biotechnology for modifying a plant's traits.

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The present invention provides novel transcription factors useful for modifying a plant's phenotype in desirable ways, such as modifying a plant's environmental stress tolerance.

SUMMARY OF THE INVENTION

In a first aspect, the invention relates to a recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-27, or a complementary nucleotide sequence thereof; (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a); (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-27, or a

complementary nucleotide sequence thereof; (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c); (e) a nucleotide sequence which hybridizes under stringent conditions over substantially the entire length of a nucleotide sequence of one or more of: (a), (b), (c), or (d); (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e); (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide having a biological activity that modifies a plant's environmental stress tolerance; (h) a nucleotide sequence having at least 30% sequence identity to a nucleotide sequence of any of (a)-(g); (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g); (j) a nucleotide sequence which encodes a polypeptide having at least 30% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27; (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27; and (I) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-27. The recombinant polynucleotide may further comprise a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence. The invention also relates to compositions comprising at least two of the above described polynucleotides.

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In a second aspect, the invention is an isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide described above.

In another aspect, the invention is a transgenic plant comprising one or more of the above described recombinant polynucleotides. In yet another aspect, the invention is a plant with altered expression levels of a polynucleotide described above or a plant with altered expression or activity levels of an above described polypeptide. Further, the invention may be a plant lacking a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-27.

The plant may be a soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf, banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, or vegetable brassicas plant.

In a further aspect, the invention relates to a cloning or expression vector comprising the isolated or recombinant polynucleotide described above or cells comprising the cloning or expression vector.

In yet a further aspect, the invention relates to a composition produced by incubating a polynucleotide of the invention with a nuclease, a restriction enzyme, a polymerase; a polymerase and a primer; a cloning vector, or with a cell.

Furthermore, the invention relates to a method for producing a plant having improved environmental stress tolerance. The method comprises altering the expression of an isolated or recombinant polynucleotide of the invention or altering the expression or activity of a polypeptide of the invention in a plant to produce a modified plant, and selecting the modified plant for modified environmental stress tolerance.

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In another aspect, the invention relates to a method of identifying a factor that is modulated by or interacts with a polypeptide encoded by a polynucleotide of the invention. The method comprises expressing a polypeptide encoded by the polynucleotide in a plant; and identifying at least one factor that is modulated by or interacts with the polypeptide. In one embodiment the method for identifying modulating or interacting factors is by detecting binding by the polypeptide to a promoter sequence, or by detecting interactions between an additional protein and the polypeptide in a yeast two hybrid system, or by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.

In yet another aspect, the invention is a method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest. The method comprises placing the molecule in contact with a plant comprising the polynucleotide or polypeptide encoded by the polynucleotide of the invention and monitoring one or more of the expression level of the polynucleotide in the plant, the expression level of the polypeptide in the plant, and modulation of an activity of the polypeptide in the plant.

In yet another aspect, the invention relates to an integrated system, computer or computer readable medium comprising one or more character strings corresponding to a polynucleotide of the invention, or to a polypeptide encoded by the polynucleotide. The integrated system, computer or computer readable medium may comprise a link between one or more sequence strings to a modified plant environmental stress tolerance phenotype.

In yet another aspect, the invention is a method for identifying a sequence similar or homologous to one or more polynucleotides of the invention, or one or more polypeptides encoded by the polynucleotides. The method comprises providing a sequence database; and, querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.

The method may further comprise of linking the one or more of the polynucleotides of the invention, or encoded polypeptides, to a modified plant environmental stress tolerance phenotype.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a table of exemplary polynucleotide and polypeptide sequences of the invention. The table includes from left to right for each sequence: the SEQ ID No., the internal code reference number (GID), whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

Figure 2 provides a table of exemplary sequences that are homologous to other sequences provided in the Sequence Listing and that are derived from *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), identification of the homologous sequence, whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

Figure 3 provides a table of exemplary sequences that are homologous to the sequences provided in Figures 1 and 2 and that are derived from plants other than *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), the unique GenBank sequence ID No. (NID), the probability that the comparison was generated by chance (P-value), and the species from which the homologous gene was identified.

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DETAILED DESCRIPTION

The present invention relates to polynucleotides and polypeptides, e.g. for modifying phenotypes of plants.

In particular, the polynucleotides or polypeptides are useful for modifying traits associated with a plant's environmental stress tolerance when the expression levels of the polynucleotides or expression levels or activity levels of the polypeptides are altered. Specifically, the polynucleotides and polypeptides are useful for modifying traits associated with a plant's environmental stress tolerance, such as freezing, chilling, heat, drought, water saturation, salt, photoconditions, radiation and ozone, or the like. Plants with altered expression of the polynucleotides or polypeptides of the invention are more tolerant to these environmental stresses compared with plants without altered expression levels.

The polynucleotides of the invention encode plant transcription factors. The plant transcription factors are derived, e.g., from *Arabidopsis thaliana* and can belong, e.g., to one

or more of the following transcription factor families: the AP2 (APETALA2) domain transcription factor family (Riechmann and Meyerowitz (1998) J. Biol. Chem. 379:633-646); the MYB transcription factor family (Martin and Paz-Ares (1997) Trends Genet. 13:67-73); the MADS domain transcription factor family (Riechmann and Meyerowitz (1997) J. Biol. Chem. 378:1079-1101); the WRKY protein family (Ishiguro and Nakamura (1994) Mol. Gen. Genet. 244:563-571); the ankyrin-repeat protein family (Zhang et al. (1992) Plant Cell 4:1575-1588); the miscellaneous protein (MISC) family (Kim et al. (1997) Plant J. 11:1237-1251); the zinc finger protein (Z) family (Klug and Schwabe (1995) FASEB J. 9: 597-604); the homeobox (HB) protein family (Duboule (1994) Guidebook to the Homeobox Genes, Oxford University Press); the CAAT-element binding proteins (Forsburg and Guarente (1989) Genes Dev. 3:1166-1178); the squamosa promoter binding proteins (SPB) (Klein et al. (1996) Mol. Gen. Genet, 1996 250:7-16); the NAM protein family; the IAA/AUX proteins (Rouse et al. (1998) Science 279:1371-1373); the HLH/MYC protein family (Littlewood et al. (1994) Prot. Profile 1:639-709); the DNA-binding protein (DBP) family (Tucker et al. (1994) EMBO J. 13:2994-3002); the bZIP family of transcription factors (Foster et al. (1994) FASEB J. 8:192-200); the BPF-1 protein (Box P-binding factor) family (da Costa e Silva et al. (1993) Plant J. 4:125-135); and the golden protein (GLD) family (Hall et al. (1998) Plant Cell 10:925-936).

In addition to methods for modifying a plant phenotype by employing one or more polynucleotides and polypeptides of the invention described herein, the polynucleotides and polypeptides of the invention have a variety of additional uses. These uses include their use in the recombinant production (i.e, expression) of proteins; as regulators of plant gene expression, as diagnostic probes for the presence of complementary or partially complementary nucleic acids (including for detection of natural coding nucleic acids); as substrates for further reactions, e.g., mutation reactions, PCR reactions, or the like, of as substrates for cloning e.g., including digestion or ligation reactions, and for identifying exogenous or endogenous modulators of the transcription factors.

DEFINITIONS

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A "polynucleotide" is a nucleic acid sequence comprising a plurality of polymerized nucleotide residues, e.g., at least about 15 consecutive polymerized nucleotide residues, optionally at least about 30 consecutive nucleotides, at least about 50 consecutive nucleotides. In many instances, a polynucleotide comprises a nucleotide sequence encoding a polypeptide (or protein) or a domain or fragment thereof. Additionally, the polynucleotide may comprise a promoter, an intron, an enhancer region, a polyadenylation site, a translation initiation

site, 5' or 3' untranslated regions, a reporter gene, a selectable marker, or the like. The polynucleotide can be single stranded or double stranded DNA or RNA. The polynucleotide optionally comprises modified bases or a modified backbone. The polynucleotide can be, e.g., genomic DNA or RNA, a transcript (such as an mRNA), a cDNA, a PCR product, a cloned DNA, a synthetic DNA or RNA, or the like. The polynucleotide can comprise a sequence in either sense or antisense orientations.

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A "recombinant polynucleotide" is a polynucleotide that is not in its native state, e.g., the polynucleotide comprises a nucleotide sequence not found in nature, or the polynucleotide is in a context other than that in which it is naturally found, e.g., separated from nucleotide sequences with which it typically is in proximity in nature, or adjacent (or contiguous with) nucleotide sequences with which it typically is not in proximity. For example, the sequence at issue can be cloned into a vector, or otherwise recombined with one or more additional nucleic acid.

An "isolated polynucleotide" is a polynucleotide whether naturally occurring or recombinant, that is present outside the cell in which it is typically found in nature, whether purified or not. Optionally, an isolated polynucleotide is subject to one or more enrichment or purification procedures, e.g., cell lysis, extraction, centrifugation, precipitation, or the like.

A "recombinant polypeptide" is a polypeptide produced by translation of a recombinant polypucleotide. An "isolated polypeptide," whether a naturally occurring or a recombinant polypeptide, is more enriched in (or out of) a cell than the polypeptide in its natural state in a wild type cell, e.g., more than about 5% enriched, more than about 10% enriched, or more than about 20%, or more than about 50%, or more, enriched, i.e., alternatively denoted: 105%, 110%, 120%, 150% or more, enriched relative to wild type standardized at 100%. Such an enrichment is not the result of a natural response of a wild type plant. Alternatively, or additionally, the isolated polypeptide is separated from other cellular components with which it is typically associated, e.g., by any of the various protein purification methods herein.

The term "transgenic plant" refers to a plant that contains genetic material, not found in a wild type plant of the same species, variety or cultivar. The genetic material may include a transgene, an insertional mutagenesis event (such as by transposon or T-DNA insertional mutagenesis), an activation tagging sequence, a mutated sequence, a homologous recombination event or a sequence modified by chimeraplasty. Typically, the foreign genetic material has been introduced into the plant by human manipulation.

A transgenic plant may contain an expression vector or cassette. The expression cassette typically comprises a polypeptide-encoding sequence operably linked (i.e., under

regulatory control of) to appropriate inducible or constitutive regulatory sequences that allow for the expression of polypeptide. The expression cassette can be introduced into a plant by transformation or by breeding after transformation of a parent plant. A plant refers to a whole plant as well as to a plant part, such as seed, fruit, leaf, or root, plant tissue, plant cells or any other plant material, e.g., a plant explant, as well as to progeny thereof, and to *in vitro* systems that mimic biochemical or cellular components or processes in a cell.

The phrase "ectopically expression or altered expression" in reference to a polynucleotide indicates that the pattern of expression in, e.g., a transgenic plant or plant tissue, is different from the expression pattern in a wild type plant or a reference plant of the same species. For example, the polynucleotide or polypeptide is expressed in a cell or tissue type other than a cell or tissue type in which the sequence is expressed in the wild type plant, or by expression at a time other than at the time the sequence is expressed in the wild type plant, or by a response to different inducible agents, such as hormones or environmental signals, or at different expression levels (either higher or lower) compared with those found in a wild type plant. The term also refers to altered expression patterns that are produced by lowering the levels of expression to below the detection level or completely abolishing expression. The resulting expression pattern can be transient or stable, constitutive or inducible. In reference to a polypeptide, the term "ectopic expression or altered expression" further may relate to altered activity levels resulting from the interactions of the polypeptides with exogenous or endogenous modulators or from interactions with factors or as a result of the chemical modification of the polypeptides.

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The term "fragment" or "domain," with respect to a polypeptide, refers to a subsequence of the polypeptide. In some cases, the fragment or domain, is a subsequence of the polypeptide which performs at least one biological function of the intact polypeptide in substantially the same manner, or to a similar extent, as does the intact polypeptide. For example, a polypeptide fragment can comprise a recognizable structural motif or functional domain such as a DNA binding domain that binds to a DNA promoter region, an activation domain or a domain for protein-protein interactions. Fragments can vary in size from as few as 6 amino acids to the full length of the intact polypeptide, but are preferably at least about 30 amino acids in length and more preferably at least about 60 amino acids in length. In reference to a nucleotide sequence, "a fragment" refers to any subsequence of a polynucleotide, typically, of at least consecutive about 15 nucleotides, preferably at least about 30 nucleotides, more preferably at least about 50, of any of the sequences provided herein.

The term "trait" refers to a physiological, morphological, biochemical or physical characteristic of a plant or particular plant material or cell. In some instances, this characteristic

is visible to the human eye, such as seed or plant size, or can be measured by available biochemical techniques, such as the protein, starch or oil content of seed or leaves or by the observation of the expression level of genes, e.g., by employing Northern analysis, RT-PCR, microarray gene expression assays or reporter gene expression systems, or by agricultural observations such as stress tolerance, yield or pathogen tolerance.

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"Trait modification" refers to a detectable difference in a characteristic in a plant ectopically expressing a polynucleotide or polypeptide of the present invention relative to a plant not doing so, such as a wild type plant. In some cases, the trait modification can be evaluated quantitatively. For example, the trait modification can entail at least about a 2% increase or decrease in an observed trait (difference), at least a 5% difference, at least about a 10% difference, at least about a 20% difference, at least about a 30%, at least about a 50%, at least about a 70%, or at least about a 100%, or an even greater difference. It is known that there can be a natural variation in the modified trait. Therefore, the trait modification observed entails a change of the normal distribution of the trait in the plants compared with the distribution observed in wild type plant.

Trait modifications of particular interest include those to seed (such as embryo or endosperm), fruit, root, flower, leaf, stem, shoot, seedling or the like, including: enhanced tolerance to environmental conditions including freezing, chilling, heat, drought, water saturation, radiation and ozone; improved tolerance to microbial, fungal or viral diseases; improved tolerance to pest infestations, including nematodes, mollicutes, parasitic higher plants or the like; decreased herbicide sensitivity; improved tolerance of heavy metals or enhanced ability to take up heavy metals; improved growth under poor photoconditions (e.g., low light and/or short day length), or changes in expression levels of genes of interest. Other phenotype that can be modified relate to the production of plant metabolites, such as variations in the production of taxol, tocopherol, tocotrienol, sterols, phytosterols, vitamins, wax monomers, anti-oxidants, amino acids, lignins, cellulose, tannins, prenyllipids (such as chlorophylls and carotenoids), glucosinolates, and terpenoids, enhanced or compositionally altered protein or oil production (especially in seeds), or modified sugar (insoluble or soluble) and/or starch composition. Physical plant characteristics that can be modified include cell development (such as the number of trichomes), fruit and seed size and number, yields of plant parts such as stems, leaves and roots, the stability of the seeds during storage, characteristics of the seed pod (e.g., susceptibility to shattering), root hair length and quantity, internode distances, or the quality of seed coat. Plant growth characteristics that can be modified include growth rate, germination rate of seeds, vigor of plants and seedlings, leaf and flower senescence, male sterility, apomixis, flowering time,

flower abscission, rate of nitrogen uptake, biomass or transpiration characteristics, as well as plant architecture characteristics such as apical dominance, branching patterns, number of organs, organ identity, organ shape or size.

POLYPEPTIDES AND POLYNUCLEOTIDES OF THE INVENTION

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The present invention provides, among other things, transcription factors (TFs), and transcription factor homologue polypeptides, and isolated or recombinant polynucleotides encoding the polypeptides. These polypeptides and polynucleotides may be employed to modify a plant's environmental stress tolerance.

Exemplary polynucleotides encoding the polypeptides of the invention were identified in the *Arabidopsis thaliana* GenBank database using publicly available sequence analysis programs and parameters. Sequences initially identified were then further characterized to identify sequences comprising specified sequence strings corresponding to sequence motifs present in families of known transcription factors. Polynucleotide sequences meeting such criteria were confirmed as transcription factors.

Additional polynucleotides of the invention were identified by screening Arabidopsis thaliana and/or other plant cDNA libraries with probes corresponding to known transcription factors under low stringency hybridization conditions. Additional sequences, including full length coding sequences were subsequently recovered by the rapid amplification of cDNA ends (RACE) procedure, using a commercially available kit according to the manufacturer's instructions. Where necessary, multiple rounds of RACE are performed to isolate 5' and 3' ends. The full length cDNA was then recovered by a routine end-to-end polymerase chain reaction (PCR) using primers specific to the isolated 5' and 3' ends. Exemplary sequences are provided in the Sequence Listing.

The polynucleotides of the invention were ectopically expressed in overexpressor or knockout plants and changes in the environmental stress tolerance of the plants was observed. Therefore, the polynucleotides and polypeptides can be employed to improve the environmental stress resistance of plants.

Making polynucleotides

The polynucleotides of the invention include sequences that encode transcription factors and transcription factor homologue polypeptides and sequences complementary thereto, as well as unique fragments of coding sequence, or sequence complementary thereto. Such polynucleotides can be, e.g., DNA or RNA, e.g., mRNA, cRNA, synthetic RNA, genomic DNA, cDNA synthetic DNA, oligonucleotides, etc. The polynucleotides are either double-stranded or

PCT/US00/31458 WO 01/36598

single-stranded, and include either, or both sense (i.e., coding) sequences and antisense (i.e., noncoding, complementary) sequences. The polynucleotides include the coding sequence of a transcription factor, or transcription factor homologue polypeptide, in isolation, in combination with additional coding sequences (e.g., a purification tag, a localization signal, as a fusionprotein, as a pre-protein, or the like), in combination with non-coding sequences (e.g., introns or inteins, regulatory elements such as promoters, enhancers, terminators, and the like), and/or in a vector or host environment in which the polynucleotide encoding a transcription factor or transcription factor homologue polypeptide is an endogenous or exogenous gene.

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A variety of methods exist for producing the polynucleotides of the invention. Procedures for identifying and isolating DNA clones are well known to those of skill in the art, 10 and are described in, e.g., Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology volume 152 Academic Press, Inc., San Diego, CA ("Berger"); Sambrook et al., Molecular Cloning - A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989 ("Sambrook") and Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing 15 Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2000) ("Ausubel").

Alternatively, polynucleotides of the invention, can be produced by a variety of in vitro amplification methods adapted to the present invention by appropriate selection of specific or degenerate primers. Examples of protocols sufficient to direct persons of skill through in vitro amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Qbeta-replicase amplification and other RNA polymerase mediated techniques (e.g., NASBA), e.g., for the production of the homologous nucleic acids of the invention are found in Berger, Sambrook, and Ausubel, as well as Mullis et al., (1987) PCR Protocols A Guide to Methods and Applications (Innis et al. eds) Academic Press Inc. San Diego, CA (1990) (Innis). Improved methods for cloning in vitro amplified nucleic acids are described in Wallace et al., 25 U.S. Pat. No. 5,426,039. Improved methods for amplifying large nucleic acids by PCR are summarized in Cheng et al. (1994) Nature 369: 684-685 and the references cited therein, in which PCR amplicons of up to 40kb are generated. One of skill will appreciate that essentially any RNA can be converted into a double stranded DNA suitable for restriction digestion, PCR expansion and sequencing using reverse transcriptase and a polymerase. See, e.g., Ausubel, 30 Sambrook and Berger, all supra.

Alternatively, polynucleotides and oligonucleotides of the invention can be assembled from fragments produced by solid-phase synthesis methods. Typically, fragments of up to approximately 100 bases are individually synthesized and then enzymatically or chemically

ligated to produce a desired sequence, e.g., a polynucletotide encoding all or part of a transcription factor. For example, chemical synthesis using the phosphoramidite method is described, e.g., by Beaucage et al. (1981) <u>Tetrahedron Letters</u> 22:1859-69; and Matthes et al. (1984) <u>EMBO J.</u> 3:801-5. According to such methods, oligonucleotides are synthesized, purified, annealed to their complementary strand, ligated and then optionally cloned into suitable vectors. And if so desired, the polynucleotides and polypeptides of the invention can be custom ordered from any of a number of commercial suppliers.

HOMOLOGOUS SEQUENCES

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Sequences homologous, i.e., that share significant sequence identity or similarity, to those provided in the Sequence Listing, derived from Arabidopsis thaliana or from other plants of choice are also an aspect of the invention. Homologous sequences can be derived from any plant including monocots and dicots and in particular agriculturally important plant species. including but not limited to, crops such as soybean, wheat, corn, potato, cotton, rice, oilseed rape (including canola), sunflower, alfalfa, sugarcane and turf; or fruits and vegetables, such as banana, blackberry, blueberry, strawberry, and raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits (such as apple, peach, pear, cherry and plum) and vegetable brassicas (such as broccoli, cabbage, cauliflower, brussel sprouts and kohlrabi). Other crops, fruits and vegetables whose phenotype can be changed include barley, rye, millet, sorghum, currant, avocado, citrus fruits such as oranges, lemons, grapefruit and tangerines, artichoke, cherries, nuts such as the walnut and peanut, endive, leek, roots, such as arrowroot, beet, cassava, turnip, radish, yam, and sweet potato, and beans. The homologous sequences may also be derived from woody species, such pine, poplar and eucalyptus.

Transcription factors that are homologous to the listed sequences will typically share at least about 30% amino acid sequence identity. More closely related transcription factors can share at least about 50%, about 60%, about 65%, about 70%, about 75% or about 80% or about 90% or about 95% or about 98% or more sequence identity with the listed sequences. Factors that are most closely related to the listed sequences share, e.g., at least about 85%, about 90% or about 95% or more % sequence identity to the listed sequences. At the nucleotide level, the sequences will typically share at least about 40% nucleotide sequence identity, preferably at least about 50%, about 60%, about 70% or about 80% sequence identity, and more preferably about 85%, about 90%, about 95% or about 97% or more sequence identity to one or more of the

PCT/US00/31458 WO 01/36598

listed sequences. The degeneracy of the genetic code enables major variations in the nucleotide sequence of a polynucleotide while maintaining the amino acid sequence of the encoded protein. Conserved domains within a transcription factor family may exhibit a higher degree of sequence homology, such as at least 65% sequence identity including conservative substitutions, and preferably at least 80% sequence identity.

Identifying Nucleic Acids by Hybridization

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Polynucleotides homologous to the sequences illustrated in the Sequence Listing can be identified, e.g., by hybridization to each other under stringent or under highly stringent conditions. Single stranded polynucleotides hybridize when they associate based on a variety of well characterized physico-chemical forces, such as hydrogen bonding, solvent exclusion, base stacking and the like. The stringency of a hybridization reflects the degree of sequence identity of the nucleic acids involved, such that the higher the stringency, the more similar are the two polynucleotide strands. Stringency is influenced by a variety of factors, including temperature, salt concentration and composition, organic and non-organic additives, solvents, etc. present in both the hybridization and wash solutions and incubations (and number), as described in more detail in the references cited above.

An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is about 5°C to 20°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Nucleic acid molecules that hybridize under stringent conditions will typically hybridize to a probe based on either the entire cDNA or selected portions, e.g., to a unique subsequence, of the cDNA under wash conditions of 0.2x SSC to 2.0 x SSC, 0.1% SDS at 50-65° C, for example 0.2 x SSC, 0.1% SDS at 65° C. For identification of less closely related homologues washes can be performed at a lower temperature, e.g., 50° C. In general, stringency is increased by raising. the wash temperature and/or decreasing the concentration of SSC.

As another example, stringent conditions can be selected such that an oligonucleotide that is perfectly complementary to the coding oligonucleotide hybridizes to the . coding oligonucleotide with at least about a 5-10x higher signal to noise ratio than the ratio for hybridization of the perfectly complementary oligonucleotide to a nucleic acid encoding a transcription factor known as of the filing date of the application. Conditions can be selected such that a higher signal to noise ratio is observed in the particular assay which is used, e.g., about 15x, 25x, 35x, 50x or more. Accordingly, the subject nucleic acid hybridizes to the unique

coding oligonucleotide with at least a 2x higher signal to noise ratio as compared to hybridization of the coding oligonucleotide to a nucleic acid encoding known polypeptide. Again, higher signal to noise ratios can be selected, e.g., about 5x, 10x, 25x, 35x, 50x or more. The particular signal will depend on the label used in the relevant assay, e.g., a fluorescent label, a colorimetric label, a radio active label, or the like.

Alternatively, transcription factor homologue polypeptides can be obtained by screening an expression library using antibodies specific for one or more transcription factors. With the provision herein of the disclosed transcription factor, and transcription factor homologue nucleic acid sequences, the encoded polypeptide(s) can be expressed and purified in a heterologous expression system (e.g., *E. coli*) and used to raise antibodies (monoclonal or polyclonal) specific for the polypeptide(s) in question. Antibodies can also be raised against synthetic peptides derived from transcription factor, or transcription factor homologue, amino acid sequences. Methods of raising antibodies are well known in the art and are described in Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. Such antibodies can then be used to screen an expression library produced from the plant from which it is desired to clone additional transcription factor homologues, using the methods described above. The selected cDNAs can be confirmed by sequencing and enzymatic activity.

SEQUENCE VARIATIONS

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It will readily be appreciated by those of skill in the art, that any of a variety of polynucleotide sequences are capable of encoding the transcription factors and transcription factor homologue polypeptides of the invention. Due to the degeneracy of the genetic code, many different polynucleotides can encode identical and/or substantially similar polypeptides in addition to those sequences illustrated in the Sequence Listing.

For example, Table 1 illustrates, e.g., that the codons AGC, AGT, TCA, TCC,

TCG, and TCT all encode the same amino acid: serine. Accordingly, at each position in the sequence where there is a codon encoding serine, any of the above trinucleotide sequences can be used without altering the encoded polypeptide.

Table 1

| Amino acids | | | Codon | | | | | |
|---------------|-----|----------|-------|-----|-----|-------|-----|-----|
| Alanine | Ala | A | GCA | GCC | GCG | GCU | | |
| Cysteine | Cys | С | TGC | TGT | | | | |
| Aspartic acid | Asp | D | GAC | GAT | | | | |
| Glutamic acid | Glu | E | GAA | GAG | | | | |
| Phenylalanine | Phe | F | TTC | TTT | | | | |
| Glycine | Gly | G | GGA | GGC | GGG | GGT | | |
| Histidine | His | H | CAC | CAT | | | | |
| Isoleucine | Ile | I | ATA | ATC | ATT | | | |
| Lysine | Lys | K | AAA | AAG | | | | |
| Leucine | Leu | L | TTA | TTG | CTA | CTC - | CTG | CTT |
| Methionine | Met | M | ATG | | | | | |
| Asparagine | Asn | N | AAC | AAT | | | | |
| Proline | Pro | P | CCA | CCC | CCG | CCT | | |
| Glutamine | Gln | Q | CAA | CAG | | | | |
| Arginine | Arg | R | AGA | AGG | CGA | CGÇ | CGG | CGT |
| Serine | Ser | S | AGC | AGT | TCA | TCC | TCG | TCT |
| Threonine | Thr | T | ACA | ACC | ACG | ACT | | |
| Valine | Val | V | GTA | GTC | GTG | GTT | | |
| Tryptophan | Trp | W | TGG | | | | | |
| Tyrosine | Tyr | <u>Y</u> | TAC | TAT | | | | |

Sequence alterations that do not change the amino acid sequence encoded by the polynucleotide are termed "silent" variations. With the exception of the codons ATG and TGG, encoding methionine and tryptophan, respectively, any of the possible codons for the same amino acid can be substituted by a variety of techniques, e.g., site-directed mutagenesis, available in the art. Accordingly, any and all such variations of a sequence selected from the above table are a feature of the invention.

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In addition to silent variations, other conservative variations that alter one, or a few amino acids in the encoded polypeptide, can be made without altering the function of the polypeptide, these conservative variants are, likewise, a feature of the invention.

For example, substitutions, deletions and insertions introduced into the sequences provided in the Sequence Listing are also envisioned by the invention. Such sequence modifications can be engineered into a sequence by site-directed mutagenesis (Wu (ed.) Meth. Enzymol. (1993) vol. 217, Academic Press) or the other methods noted below. Amino acid substitutions are typically of single residues; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. In preferred embodiments, deletions or insertions are made in adjacent pairs, e.g., a deletion of two residues or insertion of two residues. Substitutions, deletions, insertions or any combination thereof can be

combined to arrive at a sequence. The mutations that are made in the polynucleotide encoding the transcription factor should not place the sequence out of reading frame and should not create complementary regions that could produce secondary mRNA structure. Preferably, the polypeptide encoded by the DNA performs the desired function.

Conservative substitutions are those in which at least one residue in the amino acid sequence has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the Table 2 when it is desired to maintain the activity of the protein. Table 2 shows amino acids which can be substituted for an amino acid in a protein and which are typically regarded as conservative substitutions.

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Table 2

| Residue | Conservative Substitutions | | | |
|---------|----------------------------|--|--|--|
| Ala | Ser | | | |
| Arg | Lys | | | |
| - Asn | Gln; His | | | |
| Asp | Glu | | | |
| Gln | Asn | | | |
| Cys | Ser | | | |
| Glu | Asp | | | |
| Gly | Pro | | | |
| His | Asn; Gln | | | |
| Ile | Leu, Val | | | |
| Leu | Ile; Val | | | |
| Lys | Arg; Gln | | | |
| Met | Leu; Ile | | | |
| Phe | Met; Leu; Tyr | | | |
| Ser | Thr; Gly | | | |
| Thr | Ser;Val | | | |
| Trp | Тут | | | |
| Tyr | Trp; Phe | | | |
| Val | Ile; Leu | | | |

Substitutions that are less conservative than those in Table 2 can be selected by picking residues that differ more significantly in their effect on maintaining (a) the structure of

the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in protein properties will be those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

10 <u>FURTHER MODIFYING SEQUENCES OF THE INVENTION—MUTATION/ FORCED</u> EVOLUTION

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In addition to generating silent or conservative substitutions as noted, above, the present invention optionally includes methods of modifying the sequences of the Sequence Listing. In the methods, nucleic acid or protein modification methods are used to alter the given sequences to produce new sequences and/or to chemically or enzymatically modify given sequences to change the properties of the nucleic acids or proteins.

Thus, in one embodiment, given nucleic acid sequences are modified, e.g., according to standard mutagenesis or artificial evolution methods to produce modified sequences. For example, Ausubel, *supra*, provides additional details on mutagenesis methods. Artificial forced evolution methods are described, e.g., by Stemmer (1994) Nature 370:389-391, and Stemmer (1994) Proc. Natl. Acad. Sci. USA 91:10747-10751. Many other mutation and evolution methods are also available and expected to be within the skill of the practitioner.

Similarly, chemical or enzymatic alteration of expressed nucleic acids and polypeptides can be performed by standard methods. For example, sequence can be modified by addition of lipids, sugars, peptides, organic or inorganic compounds, by the inclusion of modified nucleotides or amino acids, or the like. For example, protein modification techniques are illustrated in Ausubel, *supra*. Further details on chemical and enzymatic modifications can be found herein. These modification methods can be used to modify any given sequence, or to modify any sequence produced by the various mutation and artificial evolution modification methods noted herein.

Accordingly, the invention provides for modification of any given nucleic acid by mutation, evolution, chemical or enzymatic modification, or other available methods, as well as for the products produced by practicing such methods, e.g., using the sequences herein as a starting substrate for the various modification approaches.

For example, optimized coding sequence containing codons preferred by a particular prokaryotic or eukaryotic host can be used e.g., to increase the rate of translation or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, as compared with transcripts produced using a non-optimized sequence. Translation stop codons can also be modified to reflect host preference. For example, preferred stop codons for S. cerevisiae and mammals are TAA and TGA, respectively. The preferred stop codon for monocotyledonous plants is TGA, whereas insects and E. coli prefer to use TAA as the stop codon.

The polynucleotide sequences of the present invention can also be engineered in order to alter a coding sequence for a variety of reasons, including but not limited to, alterations which modify the sequence to facilitate cloning, processing and/or expression of the gene product. For example, alterations are optionally introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to introduce splice sites, etc.

Furthermore, a fragment or domain derived from any of the polypeptides of the invention can be combined with domains derived from other transcription factors or synthetic domains to modify the biological activity of a transcription factor. For instance, a DNA binding domain derived from a transcription factor of the invention can be combined with the activation domain of another transcription factor or with a synthetic activation domain. A transcription activation domain assists in initiating transcription from a DNA binding site. Examples include the transcription activation region of VP16 or GAL4 (Moore et al. (1998) Proc. Natl. Acad. Sci. USA 95: 376-381; and Aoyama et al. (1995) Plant Cell 7:1773-1785), peptides derived from bacterial sequences (Ma and Ptashne (1987) Cell 51; 113-119) and synthetic peptides (Giniger and Ptashne, (1987) Nature 330:670-672).

EXPRESSION AND MODIFICATION OF POLYPEPTIDES

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Typically, polynucleotide sequences of the invention are incorporated into recombinant DNA (or RNA) molecules that direct expression of polypeptides of the invention in appropriate host cells, transgenic plants, in vitro translation systems, or the like. Due to the inherent degeneracy of the genetic code, nucleic acid sequences which encode substantially the same or a functionally equivalent amino acid sequence can be substituted for any listed sequence to provide for cloning and expressing the relevant homologue.

Vectors, Promoters and Expression Systems

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The present invention includes recombinant constructs comprising one or more of the nucleic acid sequences herein. The constructs typically comprise a vector, such as a plasmid, a cosmid, a phage, a virus (e.g., a plant virus), a bacterial artificial chromosome (BAC), a yeast artificial chromosome (YAC), or the like, into which a nucleic acid sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available.

General texts which describe molecular biological techniques useful herein, including the use and production of vectors, promoters and many other relevant topics, include Berger, Sambrook and Ausubel, *supra*. Any of the identified sequences can be incorporated into a cassette or vector, e.g., for expression in plants. A number of expression vectors suitable for stable transformation of plant cells or for the establishment of transgenic plants have been described including those described in Weissbach and Weissbach, (1989) Methods for Plant Molecular Biology, Academic Press, and Gelvin et al., (1990) Plant Molecular Biology Manual, Kluwer Academic Publishers. Specific examples include those derived from a Ti plasmid of Agrobacterium tumefaciens, as well as those disclosed by Herrera-Estrella et al. (1983) Nature 303: 209, Bevan (1984) Nucl Acid Res. 12: 8711-8721, Klee (1985) Bio/Technology 3: 637-642, for dicotyledonous plants.

Alternatively, non-Ti vectors can be used to transfer the DNA into monocotyledonous plants and cells by using free DNA delivery techniques. Such methods can involve, for example, the use of liposomes, electroporation, microprojectile bombardment, silicon carbide whiskers, and viruses. By using these methods transgenic plants such as wheat, rice (Christou (1991) Bio/Technology 9: 957-962) and corn (Gordon-Kamm (1990) Plant Cell 2: 603-618) can be produced. An immature embryo can also be a good target tissue for monocots for direct DNA delivery techniques by using the particle gun (Weeks et al. (1993) Plant Physiol 102: 1077-1084; Vasil (1993) Bio/Technology 10: 667-674; Wan and Lemeaux (1994) Plant Physiol 104: 37-48, and for Agrobacterium-mediated DNA transfer (Ishida et al. (1996) Nature Biotech 14: 745-750).

Typically, plant transformation vectors include one or more cloned plant coding sequence (genomic or cDNA) under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant transformation vectors typically also contain a promoter (e.g., a regulatory region controlling inducible or constitutive, environmentally-or

developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, an RNA processing signal (such as intron splice sites), a transcription termination site, and/or a polyadenylation signal.

Examples of constitutive plant promoters which can be useful for expressing the

TF sequence include: the cauliflower mosaic virus (CaMV) 35S promoter, which confers
constitutive, high-level expression in most plant tissues (see, e.g., Odel et al. (1985) Nature
313:810); the nopaline synthase promoter (An et al. (1988) Plant Physiol 88:547); and the
octopine synthase promoter (Fromm et al. (1989) Plant Cell 1: 977).

A variety of plant gene promoters that regulate gene expression in response to 10 environmental, hormonal, chemical, developmental signals, and in a tissue-active manner can be used for expression of a TF sequence in plants. Choice of a promoter is based largely on the phenotype of interest and is determined by such factors as tissue (e.g., seed, fruit, root, pollen, vascular tissue, flower, carpel, etc.), inducibility (e.g., in response to wounding, heat, cold, drought, light, pathogens, etc.), timing, developmental stage, and the like. Numerous known promoters have been characterized and can favorable be employed to promote expression of a 15 polynucleotide of the invention in a transgenic plant or cell of interest. For example, tissue specific promoters include: seed-specific promoters (such as the napin, phaseolin or DC3 promoter described in US Pat. No. 5,773,697), fruit-specific promoters that are active during fruit ripening (such as the dru 1 promoter (US Pat. No. 5,783,393), or the 2A11 promoter (US Pat. No. 20 4,943,674) and the tomato polygalacturonase promoter (Bird et al. (1988) Plant Mol Biol 11:651), root-specific promoters, such as those disclosed in US Patent Nos. 5,618,988, 5,837,848 and 5,905,186, pollen-active promoters such as PTA29, PTA26 and PTA13 (US Pat. No. 5,792,929), promoters active in vascular tissue (Ringli and Keller (1998) Plant Mol Biol 37:977-988), flowerspecific (Kaiser et al, (1995) Plant Mol Biol 28:231-243), pollen (Baerson et al. (1994) Plant Mol 25 Biol 26:1947-1959), carpels (Ohl et al. (1990) Plant Cell 2:837-848), pollen and ovules (Baerson et al. (1993) Plant Mol Biol 22:255-267), auxin-inducible promoters (such as that described in van der Kop et al. (1999) Plant Mol Biol 39:979-990 or Baumann et al. (1999) Plant Cell 11:323-334), cytokinin-inducible promoter (Guevara-Garcia (1998) Plant Mol Biol 38:743-753), promoters responsive to gibberellin (Shi et al. (1998) Plant Mol Biol 38:1053-1060, Willmott et al. (1998) 38:817-825) and the like. Additional promoters are those that elicit expression in 30 response to heat (Ainley et al. (1993) Plant Mol Biol 22: 13-23), light (e.g., the pea rbcS-3A promoter, Kuhlemeier et al. (1989) Plant Cell 1:471, and the maize rbcS promoter, Schaffner and Sheen (1991) Plant Cell 3: 997); wounding (e.g., wunl, Siebertz et al. (1989) Plant Cell 1: 961); pathogens (such as the PR-1 promoter described in Buchel et al. (1999) Plant Mol. Biol. 40:387-

396, and the PDF1.2 promoter described in Manners et al. (1998) <u>Plant Mol. Biol.</u> 38:1071-80), and chemicals such as methyl jasmonate or salicylic acid (Gatz et al. (1997) <u>Plant Mol Biol</u> 48: 89-108). In addition, the timing of the expression can be controlled by using promoters such as those acting at senescence (An and Amazon (1995) <u>Science</u> 270: 1986-1988); or late seed development (Odell et al. (1994) <u>Plant Physiol</u> 106:447-458).

Plant expression vectors can also include RNA processing signals that can be positioned within, upstream or downstream of the coding sequence. In addition, the expression vectors can include additional regulatory sequences from the 3'-untranslated region of plant genes, e.g., a 3' terminator region to increase mRNA stability of the mRNA, such as the PI-II terminator region of potato or the octopine or nopaline synthase 3' terminator regions.

Additional Expression Elements

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Specific initiation signals can aid in efficient translation of coding sequences. These signals can include, e.g., the ATG initiation codon and adjacent sequences. In cases where a coding sequence, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence (e.g., a mature protein coding sequence), or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon can be separately provided. The initiation codon is provided in the correct reading frame to facilitate transcription. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers appropriate to the cell system in use.

Expression Hosts

The present invention also relates to host cells which are transduced with vectors of the invention, and the production of polypeptides of the invention (including fragments thereof) by recombinant techniques. Host cells are genetically engineered (i.e, nucleic acids are introduced, e.g., transduced, transformed or transfected) with the vectors of this invention, which may be, for example, a cloning vector or an expression vector comprising the relevant nucleic acids herein. The vector is optionally a plasmid, a viral particle, a phage, a naked nucleic acids, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants, or amplifying the relevant gene. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art and in the references cited herein, including, Sambrook and Ausubel.

The host cell can be a eukaryotic cell, such as a yeast cell, or a plant cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Plant protoplasts are also suitable for some applications. For example, the DNA fragments are introduced into plant tissues, cultured plant cells or plant protoplasts by standard methods including electroporation (Fromm et al., (1985) Proc. Natl. Acad. Sci. USA 82, 5824, infection by viral vectors such as cauliflower mosaic virus (CaMV) (Hohn et al., (1982) Molecular Biology of Plant Tumors, (Academic Press, New York) pp. 549-560; US 4,407,956), high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface (Klein et al., (1987) Nature 327, 70-73), use of pollen as vector (WO 85/01856), or use of Agrobacterium tumefaciens or A. rhizogenes carrying a T-DNA plasmid in which DNA fragments are cloned. The T-DNA plasmid is transmitted to plant cells upon infection by Agrobacterium tumefaciens, and a portion is stably integrated into the plant genome (Horsch et al. (1984) Science 233:496-498; Fraley et al. (1983) Proc. Natl. Acad. Sci. USA 80, 4803).

The cell can include a nucleic acid of the invention which encodes a polypeptide, wherein the cells expresses a polypeptide of the invention. The cell can also include vector sequences, or the like. Furthermore, cells and transgenic plants which include any polypeptide or nucleic acid above or throughout this specification, e.g., produced by transduction of a vector of the invention, are an additional feature of the invention.

For long-term, high-yield production of recombinant proteins, stable expression can be used. Host cells transformed with a nucleotide sequence encoding a polypeptide of the invention are optionally cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein or fragment thereof produced by a recombinant cell may be secreted, membrane-bound, or contained intracellularly, depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides encoding mature proteins of the invention can be designed with signal sequences which direct secretion of the mature polypeptides through a prokaryotic or eukaryotic cell membrane.

Modified Amino Acids

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Polypeptides of the invention may contain one or more modified amino acids.

The presence of modified amino acids may be advantageous in, for example, increasing polypeptide half-life, reducing polypeptide antigenicity or toxicity, increasing polypeptide storage stability, or the like. Amino acid(s) are modified, for example, co-translationally or post-translationally during recombinant production or modified by synthetic or chemical means.

Non-limiting examples of a modified amino acid include incorporation or other use of acetylated amino acids, glycosylated amino acids, sulfated amino acids, prenylated (e.g., farnesylated, geranylgeranylated) amino acids, PEG modified (e.g., "PEGylated") amino acids, biotinylated amino acids, carboxylated amino acids, phosphorylated amino acids, etc. References adequate to guide one of skill in the modification of amino acids are replete throughout the literature.

IDENTIFICATION OF ADDITIONAL FACTORS

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A transcription factor provided by the present invention can also be used to identify additional endogenous or exogenous molecules that can affect a phentoype or trait of interest. On the one hand, such molecules include organic (small or large molecules) and/or inorganic compounds that affect expression of (i.e., regulate) a particular transcription factor. Alternatively, such molecules include endogenous molecules that are acted upon either at a transcriptional level by a transcription factor of the invention to modify a phenotype as desired. For example, the transcription factors can be employed to identify one or more downstream gene with which is subject to a regulatory effect of the transcription factor. In one approach, a transcription factor or transcription factor homologue of the invention is expressed in a host cell, e.g, a transgenic plant cell, tissue or explant, and expression products, either RNA or protein, of likely or random targets are monitored, e.g., by hybridization to a microarray of nucleic acid probes corresponding to genes expressed in a tissue or cell type of interest, by two-dimensional gel electrophoresis of protein products, or by any other method known in the art for assessing expression of gene products at the level of RNA or protein. Alternatively, a transcription factor of the invention can be used to identify promoter sequences (i.e., binding sites) involved in the regulation of a downstream target. After identifying a promoter sequence, interactions between the transcription factor and the promoter sequence can be modified by changing specific nucleotides in the promoter sequence or specific amino acids in the transcription factor that interact with the promoter sequence to alter a plant trait. Typically, transcription factor DNA binding sites are identified by gel shift assays. After identifying the promoter regions, the promoter region sequences can be employed in double-stranded DNA arrays to identify molecules that affect the interactions of the transcription factors with their promoters (Bulyk et al. (1999) Nature Biotechnology 17:573-577).

The identified transcription factors are also useful to identify proteins that modify the activity of the transcription factor. Such modification can occur by covalent modification, such as by phosphorylation, or by protein-protein (homo or-heteropolymer) interactions. Any

method suitable for detecting protein-protein interactions can be employed. Among the methods that can be employed are co-immunoprecipitation, cross-linking and co-purification through gradients or chromatographic columns, and the two-hybrid yeast system.

The two-hybrid system detects protein interactions in vivo and is described in Chien, et al., (1991), Proc. Natl. Acad. Sci. USA 88, 9578-9582 and is commercially available from Clontech (Palo Alto, Calif.). In such a system, plasmids are constructed that encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to the TF polypeptide and the other consists of the transcription activator protein's activation domain fused to an unknown protein that is encoded by a cDNA that has been recombined into the plasmid as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast Saccharomyces cerevisiae that contains a reporter gene (e.g., lacZ) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of the reporter gene. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product. Then, the library plasmids responsible for reporter gene expression are isolated and sequenced to identify the proteins encoded by the library plasmids. After identifying proteins that interact with the transcription factors, assays for compounds that interfere with the TF protein-protein interactions can be preformed.

20 <u>IDENTIFICATION OF MODULATORS</u>

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In addition to the intracellular molecules described above, extracellular molecules that alter activity or expression of a transcription factor, either directly or indirectly, can be identified. For example, the methods can entail first placing a candidate molecule in contact with a plant or plant cell. The molecule can be introduced by topical administration, such as spraying or soaking of a plant, and then the molecule's effect on the expression or activity of the TF polypeptide or the expression of the polynucleotide monitored. Changes in the expression of the TF polypeptide can be monitored by use of polyclonal or monoclonal antibodies, gel electrophoresis or the like. Changes in the expression of the corresponding polynucleotide sequence can be detected by use of microarrays, Northerns, quantitative PCR, or any other technique for monitoring changes in mRNA expression. These techniques are exemplified in Ausubel et al. (eds) <u>Current Protocols in Molecular Biology</u>, John Wiley & Sons (1998). Such changes in the expression levels can be correlated with modified plant traits and thus identified

molecules can be useful for soaking or spraying on fruit, vegetable and grain crops to modify traits in plants.

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Essentially any available composition can be tested for modulatory activity of expression or activity of any nucleic acid or polypeptide herein. Thus, available libraries of compounds such as chemicals, polypeptides, nucleic acids and the like can be tested for modulatory activity. Often, potential modulator compounds can be dissolved in aqueous or organic (e.g., DMSO-based) solutions for easy delivery to the cell or plant of interest in which the activity of the modulator is to be tested. Optionally, the assays are designed to screen large modulator composition libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays).

In one embodiment, high throughput screening methods involve providing a combinatorial library containing a large number of potential compounds (potential modulator compounds). Such "combinatorial chemical libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as target compounds.

A combinatorial chemical library can be, e.g., a collection of diverse chemical compounds generated by chemical synthesis or biological synthesis. For example, a combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (e.g., in one example, amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound of a set length). Exemplary libraries include peptide libraries, nucleic acid libraries, antibody libraries (see, e.g., Vaughn et al. (1996) Nature Biotechnology, 14(3):309-314 and PCT/US96/10287), carbohydrate libraries (see, e.g., Liang et al. Science (1996) 274:1520-1522 and U.S. Patent 5,593,853), peptide nucleic acid libraries (see, e.g., U.S. Patent 5,539,083), and small organic molecule libraries (see, e.g., benzodiazepines, Baum C&EN Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337) and the like.

Preparation and screening of combinatorial or other libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., U.S. Patent 5,010,175, Furka, Int. J. Pept. Prot. Res. 37:487-493 (1991) and Houghton et al. Nature 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used.

In addition, as noted, compound screening equipment for high-throughput screening is generally available, e.g., using any of a number of well known robotic systems that have also been developed for solution phase chemistries useful in assay systems. These systems include automated workstations including an automated synthesis apparatus and robotic systems utilizing robotic arms. Any of the above devices are suitable for use with the present invention, e.g., for high-throughput screening of potential modulators. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art.

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Indeed, entire high throughput screening systems are commercially available.

These systems typically automate entire procedures including all sample and reagent pipetting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. These configurable systems provide high throughput and rapid start up as well as a high degree of flexibility and customization. Similarly, microfluidic implementations of screening are also commercially available.

The manufacturers of such systems provide detailed protocols the various high throughput. Thus, for example, Zymark Corp. provides technical bulletins describing screening systems for detecting the modulation of gene transcription, ligand binding, and the like. The integrated systems herein, in addition to providing for sequence alignment and, optionally, synthesis of relevant nucleic acids, can include such screening apparatus to identify modulators that have an effect on one or more polynucleotides or polypeptides according to the present invention.

In some assays it is desirable to have positive controls to ensure that the components of the assays are working properly. At least two types of positive controls are appropriate. That is, known transcriptional activators or inhibitors can be incubated with cells/plants/ etc. in one sample of the assay, and the resulting increase/decrease in transcription can be detected by measuring the resulting increase in RNA/ protein expression, etc., according to the methods herein. It will be appreciated that modulators can also be combined with transcriptional activators or inhibitors to find modulators which inhibit transcriptional activation or transcriptional repression. Either expression of the nucleic acids and proteins herein or any additional nucleic acids or proteins activated by the nucleic acids or proteins herein, or both, can be monitored.

In an embodiment, the invention provides a method for identifying compositions that modulate the activity or expression of a polynucleotide or polypeptide of the invention. For example, a test compound, whether a small or large molecule, is placed in contact with a cell,

plant (or plant tissue or explant), or composition comprising the polynucleotide or polypeptide of interest and a resulting effect on the cell, plant, (or tissue or explant) or composition is evaluated by monitoring, either directly or indirectly, one or more of: expression level of the polynucleotide or polypeptide, activity (or modulation of the activity) of the polynucleotide or polypeptide. In some cases, an alteration in a plant phenotype can be detected following contact of a plant (or plant cell, or tissue or explant) with the putative modulator, e.g., by modulation of expression or activity of a polynucleotide or polypeptide of the invention.

SUBSEQUENCES

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Also contemplated are uses of polynucleotides, also referred to herein as oligonucleotides, typically having at least 12 bases, preferably at least 15, more preferably at least 20, 30, or 50 bases, which hybridize under at least highly stringent (or ultra-high stringent or ultra-ultra- high stringent conditions) conditions to a polynucleotide sequence described above. The polynucleotides may be used as probes, primers, sense and antisense agents, and the like,

according to methods as noted supra.

Subsequences of the polynucleotides of the invention, including polynucleotide fragments and oligonucleotides are useful as nucleic acid probes and primers. An oligonucleotide suitable for use as a probe or primer is at least about 15 nucleotides in length, more often at least about 18 nucleotides, often at least about 21 nucleotides, frequently at least about 30 nucleotides, or about 40 nucleotides, or more in length. A nucleic acid probe is useful in hybridization protocols, e.g., to identify additional polypeptide homologues of the invention, including protocols for microarray experiments. Primers can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods. See Sambrook and Ausubel, *supra*.

In addition, the invention includes an isolated or recombinant polypeptide including a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotides of the invention. For example, such polypeptides, or domains or fragments thereof, can be used as immunogens, e.g., to produce antibodies specific for the polypeptide sequence, or as probes for detecting a sequence of interest. A subsequence can range in size from about 15 amino acids in length up to and including the full length of the polypeptide.

PRODUCTION OF TRANSGENIC PLANTS

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Modification of Traits

The polynucleotides of the invention are favorably employed to produce transgenic plants with various traits, or characteristics, that have been modified in a desirable manner, e.g., to improve the environmental stress resistance of a plant. For example, alteration of expression levels or patterns (e.g., spatial or temporal expression patterns) of one or more of the transcription factors (or transcription factor homologues) of the invention, as compared with the levels of the same protein found in a wild type plant, can be used to modify a plant's traits. An illustrative example of trait modification, improved environmental stress tolerance, by altering expression levels of a particular transcription factor is described further in the Examples and the Sequence Listing.

Antisense and Cosuppression Approaches

In addition to expression of the nucleic acids of the invention as gene replacement or plant phenotype modification nucleic acids, the nucleic acids are also useful for sense and anti-sense suppression of expression, e.g., to down-regulate expression of a nucleic acid of the invention, e.g., as a further mechanism for modulating plant phenotype. That is, the nucleic acids of the invention, or subsequences or anti-sense sequences thereof, can be used to block expression of naturally occurring homologous nucleic acids. A variety of sense and anti-sense technologies are known in the art, e.g., as set forth in Lichtenstein and Nellen (1997)

Antisense Technology: A Practical Approach IRL Press at Oxford University, Oxford, England. In general, sense or anti-sense sequences are introduced into a cell, where they are optionally amplified, e.g., by transcription. Such sequences include both simple oligonucleotide sequences and catalytic sequences such as ribozymes.

For example, a reduction or elimination of expression (i.e., a "knock-out") of a transcription factor or transcription factor homologue polypeptide in a transgenic plant, e.g., to modify a plant trait, can be obtained by introducing an antisense construct corresponding to the polypeptide of interest as a cDNA. For antisense suppression, the transcription factor or homologue cDNA is arranged in reverse orientation (with respect to the coding sequence) relative to the promoter sequence in the expression vector. The introduced sequence need not be the full length cDNA or gene, and need not be identical to the cDNA or gene found in the plant type to be transformed. Typically, the antisense sequence need only be capable of hybridizing to the target gene or RNA of interest. Thus, where the introduced sequence is of shorter length, a higher degree of homology to the endogenous transcription factor sequence will be needed for effective antisense suppression. While antisense sequences of various lengths can be utilized, preferably,

the introduced antisense sequence in the vector will be at least 30 nucleotides in length, and improved antisense suppression will typically be observed as the length of the antisense sequence increases. Preferably, the length of the antisense sequence in the vector will be greater than 100 nucleotides. Transcription of an antisense construct as described results in the production of RNA molecules that are the reverse complement of mRNA molecules transcribed from the endogenous transcription factor gene in the plant cell.

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Suppression of endogenous transcription factor gene expression can also be achieved using a ribozyme. Ribozymes are RNA molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Patent No. 4,987,071 and U.S. Patent No. 5,543,508. Synthetic ribozyme sequences including antisense RNAs can be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that hybridize to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression.

Vectors in which RNA encoded by a transcription factor or transcription factor homologue cDNA is over-expressed can also be used to obtain co-suppression of a corresponding endogenous gene, e.g., in the manner described in U.S. Patent No. 5,231,020 to Jorgensen. Such co-suppression (also termed sense suppression) does not require that the entire transcription factor cDNA be introduced into the plant cells, nor does it require that the introduced sequence be exactly identical to the endogenous transcription factor gene of interest. However, as with antisense suppression, the suppressive efficiency will be enhanced as specificity of hybridization is increased, e.g., as the introduced sequence is lengthened, and/or as the sequence similarity between the introduced sequence and the endogenous transcription factor gene is increased.

Vectors expressing an untranslatable form of the transcription factor mRNA, e.g., sequences comprising one or more stop codon, or nonsense mutation) can also be used to suppress expression of an endogenous transcription factor, thereby reducing or eliminating it's activity and modifying one or more traits. Methods for producing such constructs are described in U.S. Patent No. 5,583,021. Preferably, such constructs are made by introducing a premature stop codon into the transcription factor gene. Alternatively, a plant trait can be modified by gene silencing using double-strand RNA (Sharp (1999) Genes and Development 13: 139-141).

Another method for abolishing the expression of a gene is by insertion mutagenesis using the T-DNA of Agrobacterium tumefaciens. After generating the insertion mutants, the mutants can be screened to identify those containing the insertion in a transcription factor or transcription factor homologue gene. Plants containing a single transgene insertion

event at the desired gene can be crossed to generate homozygous plants for the mutation (Koncz et al. (1992) Methods in Arabidopsis Research, World Scientific).

Alternatively, a plant phenotype can be altered by eliminating an endogenous gene, such as a transcription factor or transcription factor homologue, e.g., by homologous recombination (Kempin et al. (1997) Nature 389:802).

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A plant trait can also be modified by using the cre-lox system (for example, as described in US Pat. No. 5,658,772). A plant genome can be modified to include first and second lox sites that are then contacted with a Cre recombinase. If the lox sites are in the same orientation, the intervening DNA sequence between the two sites is excised. If the lox sites are in the opposite orientation, the intervening sequence is inverted.

The polynucleotides and polypeptides of this invention can also be expressed in a plant in the absence of an expression cassette by manipulating the activity or expression level of the endogenous gene by other means. For example, by ectopically expressing a gene by T-DNA activation tagging (Ichikawa et al. (1997) Nature 390 698-701; Kakimoto et al. (1996) Science 274: 982-985). This method entails transforming a plant with a gene tag containing multiple transcriptional enhancers and once the tag has inserted into the genome, expression of a flanking gene coding sequence becomes deregulated. In another example, the transcriptional machinery in a plant can be modified so as to increase transcription levels of a polynucleotide of the invention (See, e.g., PCT Publications WO 96/06166 and WO 98/53057 which describe the modification of the DNA binding specificity of zinc finger proteins by changing particular amino acids in the DNA binding motif).

The transgenic plant can also include the machinery necessary for expressing or altering the activity of a polypeptide encoded by an endogenous gene, for example by altering the phosphorylation state of the polypeptide to maintain it in an activated state.

Transgenic plants (or plant cells, or plant explants, or plant tissues) incorporating the polynucleotides of the invention and/or expressing the polypeptides of the invention can be produced by a variety of well established techniques as described above. Following construction of a vector, most typically an expression cassette, including a polynucleotide, e.g., encoding a transcription factor or transcription factor homologue, of the invention, standard techniques can be used to introduce the polynucleotide into a plant, a plant cell, a plant explant or a plant tissue of interest. Optionally, the plant cell, explant or tissue can be regenerated to produce a transgenic plant.

The plant can be any higher plant, including gymnosperms, monocotyledonous and dicotyledenous plants. Suitable protocols are available for *Leguminosae* (alfalfa, soybean,

clover, etc.), *Umbelliferae* (carrot, celery, parsnip), *Cruciferae* (cabbage, radish, rapeseed, broccoli, etc.), *Curcurbitaceae* (melons and cucumber), *Gramineae* (wheat, corn, rice, barley, millet, etc.), *Solanaceae* (potato, tomato, tobacco, peppers, etc.), and various other crops. See protocols described in Ammirato et al. (1984) <u>Handbook of Plant Cell Culture —Crop Species</u>. Macmillan Publ. Co. Shimamoto et al. (1989) <u>Nature</u> 338:274-276; Fromm et al. (1990) <u>Bio/Technology</u> 8:833-839; and Vasil et al. (1990) <u>Bio/Technology</u> 8:429-434.

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Transformation and regeneration of both monocotyledonous and dicotyledonous plant cells is now routine, and the selection of the most appropriate transformation technique will be determined by the practitioner. The choice of method will vary with the type of plant to be transformed; those skilled in the art will recognize the suitability of particular methods for given plant types. Suitable methods can include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; polyethylene glycol (PEG) mediated transformation; transformation using viruses; micro-injection of plant cells; micro-projectile bombardment of plant cells; vacuum infiltration; and Agrobacterium tumeficiens mediated transformation. Transformation means introducing a nucleotide sequence in a plant in a manner to cause stable or transient expression of the sequence.

Successful examples of the modification of plant characteristics by transformation with cloned sequences which serve to illustrate the current knowledge in this field of technology, and which are herein incorporated by reference, include: U.S. Patent Nos. 5,571,706; 5,677,175; 5,510,471; 5,750,386; 5,597,945; 5,589,615; 5,750,871; 5,268,526; 5,780,708; 5,538,880; 5,773,269; 5,736,369 and 5,610,042.

Following transformation, plants are preferably selected using a dominant selectable marker incorporated into the transformation vector. Typically, such a marker will confer antibiotic or herbicide resistance on the transformed plants, and selection of transformants can be accomplished by exposing the plants to appropriate concentrations of the antibiotic or herbicide.

After transformed plants are selected and grown to maturity, those plants showing a modified trait are identified. The modified trait can be any of those traits described above. Additionally, to confirm that the modified trait is due to changes in expression levels or activity of the polypeptide or polynucleotide of the invention can be determined by analyzing mRNA expression using Northern blots, RT-PCR or microarrays, or protein expression using immunoblots or Western blots or gel shift assays.

INTEGRATED SYSTEMS—SEQUENCE IDENTITY

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Additionally, the present invention may be an integrated system, computer or computer readable medium that comprises an instruction set for determining the identity of one or more sequences in a database. In addition, the instruction set can be used to generate or identify sequences that meet any specified criteria. Furthermore, the instruction set may be used to associate or link certain functional benefits, such improved environmental stress tolerance, with one or more identified sequence.

For example, the instruction set can include, e.g., a sequence comparison or other alignment program, e.g., an available program such as, for example, the Wisconsin Package Version 10.0, such as BLAST, FASTA, PILEUP, FINDPATTERNS or the like (GCG, Madision, WI). Public sequence databases such as GenBank, EMBL, Swiss-Prot and PIR or private sequence databases such as PhytoSeq (Incyte Pharmaceuticals, Palo Alto, CA) can be searched.

Alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. U.S.A. 85: 2444, by computerized implementations of these algorithms. After alignment, sequence comparisons between two (or more) polynucleotides or polypeptides are typically performed by comparing sequences of the two sequences over a comparison window to identify and compare local regions of sequence similarity. The comparison window can be a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 contiguous positions. A description of the method is provided in Ausubel et al., supra.

A variety of methods of determining sequence relationships can be used, including manual alignment and computer assisted sequence alignment and analysis. This later approach is a preferred approach in the present invention, due to the increased throughput afforded by computer assisted methods. As noted above, a variety of computer programs for performing sequence alignment are available, or can be produced by one of skill.

One example algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al. <u>J. Mol. Biol</u> 215:403-410 (1990). Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is

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referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence (and, therefore, in this context, homologous) if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, or less than about 0.01, and or even less than about 0.001. An additional example of a useful sequence alignment algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. The program can align, e.g., up to 300 sequences of a maximum length of 5,000 letters.

The integrated system, or computer typically includes a user input interface allowing a user to selectively view one or more sequence records corresponding to the one or more character strings, as well as an instruction set which aligns the one or more character strings with each other or with an additional character string to identify one or more region of sequence similarity. The system may include a link of one or more character strings with a particular

phenotype or gene function. Typically, the system includes a user readable output element which displays an alignment produced by the alignment instruction set.

The methods of this invention can be implemented in a localized or distributed computing environment. In a distributed environment, the methods may implemented on a single computer comprising multiple processors or on a multiplicity of computers. The computers can be linked, e.g. through a common bus, but more preferably the computer(s) are nodes on a network. The network can be a generalized or a dedicated local or wide-area network and, in certain preferred embodiments, the computers may be components of an intra-net or an internet.

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Thus, the invention provides methods for identifying a sequence similar or homologous to one or more polynucleotides as noted herein, or one or more target polypeptides encoded by the polynucleotides, or otherwise noted herein and may include linking or associating a given plant phenotype or gene function with a sequence. In the methods, a sequence database is provided (locally or across an inter or intra net) and a query is made against the sequence database using the relevant sequences herein and associated plant phenotypes or gene functions.

Any sequence herein can be entered into the database, before or after querying the database. This provides for both expansion of the database and, if done before the querying step, for insertion of control sequences into the database. The control sequences can be detected by the query to ensure the general integrity of both the database and the query. As noted, the query can be performed using a web browser based interface. For example, the database can be a centralized public database such as those noted herein, and the querying can be done from a remote terminal or computer across an internet or intranet.

EXAMPLES

The following examples are intended to illustrate but not limit the present invention.

25 EXAMPLE I. FULL LENGTH GENE IDENTIFICATION AND CLONING

Putative transcription factor sequences (genomic or ESTs) related to known transcription factors were identified in the *Arabidopsis thaliana* GenBank database using the tblastn sequence analysis program using default parameters and a P-value cutoff threshold of -4 or -5 or lower, depending on the length of the query sequence. Putative transcription factor sequence hits were then screened to identify those containing particular sequence strings. If the sequence hits contained such sequence strings, the sequences were confirmed as transcription factors.

Alternatively, Arabidopsis thaliana cDNA libraries derived from different tissues or treatments, or genomic libraries were screened to identify novel members of a transcription family using a low stringency hybridization approach. Probes were synthesized using gene specific primers in a standard PCR reaction (annealing temperature 60°C) and labeled with ³²P dCTP using the High Prime DNA Labeling Kit (Boehringer Mannheim). Purified radiolabelled probes were added to filters immersed in Church hybridization medium (0.5 M NaPO₄ pH 7.0, 7% SDS, 1 % w/v bovine serum albumin) and hybridized overnight at 60 °C with shaking. Filters were washed two times for 45 to 60 minutes with 1xSCC, 1% SDS at 60°C.

To identify additional sequence 5' or 3' of a partial cDNA sequence in a cDNA library, 5' and 3' rapid amplification of cDNA ends (RACE) was performed using the MarathonTM cDNA amplification kit (Clontech, Palo Alto, CA). Generally, the method entailed first isolating poly(A) mRNA, performing first and second strand cDNA synthesis to generate double stranded cDNA, blunting cDNA ends, followed by ligation of the MarathonTM Adaptor to the cDNA to form a library of adaptor-ligated ds cDNA.

Gene-specific primers were designed to be used along with adaptor specific primers for both 5' and 3' RACE reactions. Nested primers, rather than single primers, were used to increase PCR specificity. Using 5' and 3' RACE reactions, 5' and 3' RACE fragments were obtained, sequenced and cloned. The process can be repeated until 5' and 3' ends of the full-length gene were identified. Then the full-length cDNA was generated by PCR using primers specific to 5' and 3' ends of the gene by end-to-end PCR.

EXAMPLE II. CONSTRUCTION OF EXPRESSION VECTORS

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The sequence was amplified from a genomic or cDNA library using primers specific to sequences upstream and downstream of the coding region. The expression vector was pMEN20 or pMEN65, which are both derived from pMON316 (Sanders et al, (1987) Nucleic Acids Research 15:1543-58) and contain the CaMV 35S promoter to express transgenes. To clone the sequence into the vector, both pMEN20 and the amplified DNA fragment were digested separately with Sall and Notl restriction enzymes at 37° C for 2 hours. The digestion products were subject to electrophoresis in a 0.8% agarose gel and visualized by ethidium bromide staining. The DNA fragments containing the sequence and the linearized plasmid were excised and purified by using a Qiaquick gel extraction kit (Qiagen, CA). The fragments of interest were ligated at a ratio of 3:1 (vector to insert). Ligation reactions using T4 DNA ligase (New England Biolabs, MA) were carried out at 16° C for 16 hours. The ligated DNAs were transformed into

competent cells of the *E. coli* strain DH5alpha by using the heat shock method. The transformations were plated on LB plates containing 50 mg/l kanamycin (Sigma).

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Individual colonies were grown overnight in five milliliters of LB broth containing 50 mg/l kanamycin at 37° C. Plasmid DNA was purified by using Qiaquick Mini Prep kits (Qiagen, CA).

EXAMPLE III. TRANSFORMATION OF AGROBACTERIUM WITH THE EXPRESSION VECTOR

After the plasmid vector containing the gene was constructed, the vector was used to transform Agrobacterium tumefaciens cells expressing the gene products. The stock of Agrobacterium tumefaciens cells for transformation were made as described by Nagel et al. (1990) FEMS Microbiol Letts. 67: 325-328. Agrobacterium strain ABI was grown in 250 ml LB medium (Sigma) overnight at 28°C with shaking until an absorbance (A₆₀₀) of 0.5 – 1.0 was reached. Cells were harvested by centrifugation at 4,000 x g for 15 min at 4° C. Cells were then resuspended in 250 μl chilled buffer (1 mM HEPES, pH adjusted to 7.0 with KOH). Cells were centrifuged again as described above and resuspended in 125 μl chilled buffer. Cells were then centrifuged and resuspended two more times in the same HEPES buffer as described above at a volume of 100 μl and 750 μl, respectively. Resuspended cells were then distributed into 40 μl aliquots, quickly frozen in liquid nitrogen, and stored at -80°C.

above following the protocol described by Nagel et al. For each DNA construct to be transformed, 50 – 100 ng DNA (generally resuspended in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was mixed with 40 μl of Agrobacterium cells. The DNA/cell mixture was then transferred to a chilled cuvette with a 2mm electrode gap and subject to a 2.5 kV charge dissipated at 25 μF and 200 μF using a Gene Pulser II apparatus (Bio-Rad). After electroporation, cells were immediately resuspended in 1.0 ml LB and allowed to recover without antibiotic selection for 2 – 4 hours at 28°C in a shaking incubator. After recovery, cells were plated onto selective medium of LB broth containing 100 μg/ml spectinomycin (Sigma) and incubated for 24-48 hours at 28°C. Single colonies were then picked and inoculated in fresh medium. The presence of the plasmid construct was verified by PCR amplification and sequence analysis.

30 <u>EXAMPLE IV. TRANSFORMATION OF ARABIDOPSIS PLANTS WITH AGROBACTERIUM TUMEFACIENS WITH EXPRESSION VECTOR</u>

After transformation of Agrobacterium tumefaciens with plasmid vectors containing the gene, single Agrobacterium colonies were identified, propagated, and used to

transform Arabidopsis plants. Briefly, 500 ml cultures of LB medium containing 50 mg/l kanamycin were inoculated with the colonies and grown at 28° C with shaking for 2 days until an absorbance (A_{600}) of > 2.0 is reached. Cells were then harvested by centrifugation at 4,000 x g for 10 min, and resuspended in infiltration medium (1/2 X Murashige and Skoog salts (Sigma), 1 X Gamborg's B-5 vitamins (Sigma), 5.0% (w/v) sucrose (Sigma), 0.044 μ M benzylamino purine (Sigma), 200 μ l/L Silwet L-77 (Lehle Seeds) until an absorbance (A_{600}) of 0.8 was reached.

Prior to transformation, Arabidopsis thaliana seeds (ecotype Columbia) were sown at a density of ~10 plants per 4" pot onto Pro-Mix BX potting medium (Hummert International) covered with fiberglass mesh (18 mm X 16 mm). Plants were grown under continuous illumination (50-75 μE/m²/sec) at 22-23° C with 65-70% relative humidity. After about 4 weeks, primary inflorescence stems (bolts) are cut off to encourage growth of multiple secondary bolts. After flowering of the mature secondary bolts, plants were prepared for transformation by removal of all siliques and opened flowers.

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The pots were then immersed upside down in the mixture of Agrobacterium infiltration medium as described above for 30 sec, and placed on their sides to allow draining into a 1' x 2' flat surface covered with plastic wrap. After 24 h, the plastic wrap was removed and pots are turned upright. The immersion procedure was repeated one week later, for a total of two immersions per pot. Seeds were then collected from each transformation pot and analyzed following the protocol described below.

20 EXAMPLE V, IDENTIFICATION OF ARABIDOPSIS PRIMARY TRANSFORMANTS

Seeds collected from the transformation pots were sterilized essentially as follows. Seeds were dispersed into in a solution containing 0.1% (v/v) Triton X-100 (Sigma) and sterile H₂O and washed by shaking the suspension for 20 min. The wash solution was then drained and replaced with fresh wash solution to wash the seeds for 20 min with shaking. After removal of the second wash solution, a solution containing 0.1% (v/v) Triton X-100 and 70% ethanol (Equistar) was added to the seeds and the suspension was shaken for 5 min. After removal of the ethanol/detergent solution, a solution containing 0.1% (v/v) Triton X-100 and 30% (v/v) bleach (Clorox) was added to the seeds, and the suspension was shaken for 10 min. After removal of the bleach/detergent solution, seeds were then washed five times in sterile distilled H₂O. The seeds were stored in the last wash water at 4°C for 2 days in the dark before being plated onto antibiotic selection medium (1 X Murashige and Skoog salts (pH adjusted to 5.7 with 1M KOH), 1 X Gamborg's B-5 vitamins, 0.9% phytagar (Life Technologies), and 50 mg/l kanamycin). Seeds were germinated under continuous illumination (50-75 μE/m²/sec) at 22-23°

C. After 7-10 days of growth under these conditions, kanamycin resistant primary transformants (T₁ generation) were visible and obtained. These seedlings were transferred first to fresh selection plates where the seedlings continued to grow for 3-5 more days, and then to soil (Pro-Mix BX potting medium).

Primary transformants were crossed and progeny seeds (T₂) collected; kanamycin resistant seedlings were selected and analyzed. The expression levels of the recombinant polynucleotides in the transformants varies from about a 5% expression level increase to a least a 100% expression level increase. Similar observations are made with respect to polypeptide level expression.

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EXAMPLE VI. IDENTIFICATION OF ARABIDOPSIS PLANTS WITH TRANSCRIPTION FACTOR GENE KNOCKOUTS

The screening of insertion mutagenized Arabidopsis collections for null mutants
in a known target gene was essentially as described in Krysan et al (1999) Plant Cell 11:22832290. Briefly, gene-specific primers, nested by 5-250 base pairs to each other, were designed
from the 5' and 3' regions of a known target gene. Similarly, nested sets of primers were also
created specific to each of the T-DNA or transposon ends (the "right" and "left" borders). All
possible combinations of gene specific and T-DNA/transposon primers were used to detect by
PCR an insertion event within or close to the target gene. The amplified DNA fragments were
then sequenced which allows the precise determination of the T-DNA/transposon insertion point
relative to the target gene. Insertion events within the coding or intervening sequence of the
genes were deconvoluted from a pool comprising a plurality of insertion events to a single unique
mutant plant for functional characterization. The method is described in more detail in Yu and
Adam, US Application Serial No. 09/177,733 filed October 23, 1998.

EXAMPLE VII. IDENTIFICATION OF ENVIRONMENTAL STRESS TOLERANCE PHENOTYPE IN OVEREXPRESSOR OR GENE KNOCKOUT PLANTS

Experiments were performed to identify those transformants or knockouts that exhibited an improved environmental stress tolerance. For such studies, the transformants were exposed to a variety of environmental stresses. Plants were exposed to chilling stress (6 hour exposure to 4-8°C), heat stress (6 hour exposure to 32-37°C), high salt stress (6 hour exposure to 200 mM NaCl), drought stress (168 hours after removing water from trays), osmotic stress (6 hour exposure to 3 M mannitol), or nutrient limitation (nitrogen, phosphate, and potassium) (Nitrogen: all components of MS medium remained constant except N was reduced to 20mg/L of

NH 4 NO3, or Phosphate: All components of MS medium except KH 2 PO 4, which was replaced by K2SO4, Potassium: All components of MS medium except removal of KNO3 and KH2PO4, which were replaced by NaH4PO4).

Table 3 shows the phenotypes observed for particular overexpressor or knockout

plants and provides the SEQ ID No., the internal reference code (GID), whether a knockout or
overexpressor plant was analyzed and the observed phenotype.

Table 3

| SEQ ID No. | GID | Knockout (KO) or overexpressor (OX) | Phenotype observed |
|------------|------|-------------------------------------|--|
| 1 | G22 | OE | Increased tolerance to high salt |
| 3 | G188 | KO | Better germination under osmotic stress |
| 5 | G225 | OE | Increased tolerance to nitrogen-limited medium |
| 7 | G226 | OE | Increased tolerance to nitrogen-limited medium |
| 9 | G256 | OE | Better germination and growth in cold |
| 11 | G419 | OE | Increased tolerance to potassium-free medium |
| 13 | G464 | OE | Better germination and growth in heat |
| 15 | G482 | OE | Increased tolerance to high salt |
| 17 | G502 | KO | Increased sensitivity to osmotic stress |
| 19 | G526 | OE | Increased sensitivity to osmotic stress |
| 21 | G545 | OE · | Susceptible to high salt |
| 23 | G561 | OE | Increased tolerance to potassium-free medium |
| 25 | G664 | OE | Better germination and growth in cold |
| 27 | G682 | OE | Better germination and growth in heat |
| 29 | G911 | OE . | Increased growth on potassium-free medium |
| 31 | G964 | OE | Better germination and growth in heat |
| 33 | G394 | OE | More sensitive to chilling |
| 35 | G489 | OE - | Increased tolerance to osmotic stress |

For a particular overexpressor that shows a decreased tolerance to an environmental stress, it may be more useful to select a plant with a decreased expression of the particular transcription factor. For a particular knockout that shows a decreased tolerance to an environmental stress, it may be more useful to select a plant with an increased expression of the particular transcription factor.

EXAMPLE VIII. IDENTIFICATION OF HOMOLOGOUS SEQUENCES

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Homologous sequences from *Arabidopsis* and plant species other than *Arabidopsis* were identified using database sequence search tools, such as the Basic Local Alignment Search Tool (BLAST) (Altschul et al. (1990) <u>J. Mol. Biol.</u> 215:403-410; and Altschul et al. (1997) <u>Nucl. Acid Res.</u> 25: 3389-3402). The tblastx sequence analysis programs were employed using the

BLOSUM-62 scoring matrix (Henikoff, S. and Henikoff, J. G. (1992) Proc. Natl. Acad. Sci. USA 89: 10915-10919).

Identified *Arabidopsis* homologous sequences are provided in Figure 2 and included in the Sequence Listing. The percent sequence identity among these sequences is as low as 47% sequence identity. Additionally, the entire NCBI GenBank database was filtered for sequences from all plants except *Arabidopsis thaliana* by selecting all entries in the NCBI GenBank database associated with NCBI taxonomic ID 33090 (Viridiplantae; all plants) and excluding entries associated with taxonomic ID 3701 (*Arabidopsis thaliana*). These sequences were compared to sequences representing genes of SEQ IDs Nos. 1-54 on 9/26/2000 using the Washington University TBLASTX algorithm (version 2.0a19MP). For each gene of SEQ IDs Nos. 1-54, individual comparisons were ordered by probability score (P-value), where the score reflects the probability that a particular alignment occurred by chance. For example, a score of 3.6e-40 is 3.6 x 10⁻⁴⁰. For up to ten species, the gene with the lowest P-value (and therefore the most likely homolog) is listed in Figure 3.

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In addition to P-values, comparisons were also scored by percentage identity. Percentage identity reflects the degree to which two segments of DNA or protein are identical over a particular length. The ranges of percent identity between the non-Arabidopsis genes shown in Figure 3 and the Arabidopsis genes in the sequence listing are: SEQ ID No. 1: 53%-67%; SEQ ID No. 3: 38%-76%; SEQ ID No. 5: 34%-67%; SEQ ID No. 7: 50%-69%; SEQ ID No. 9: 32%-91%; SEQ ID No. 11: 48%-66%; SEQ ID No. 13: 34%-60%; SEQ ID No. 15: 58%-81%; SEQ ID No. 17: 65%-94%; SEQ ID No. 19: 72%-83%; SEQ ID No. 21: 52%-64%; SEQ ID No. 23: 40%-89%; SEQ ID No. 25: 86%-97%; SEQ ID No. 27: 41%-75%; SEQ ID No. 29: 29%-72%; SEQ ID No. 31: 49%-70%; SEQ ID No. 33: 56%-86%; SEQ ID No. 35: 61%-84%; SEQ ID No. 37: 40%-58%; SEQ ID No. 39: 63%-87%; SEQ ID No. 41: 51%-88%; SEQ ID No. 43: 80%-90%; SEQ ID No. 45: 79%-90%; SEQ ID No. 47: 30%-58%; SEQ ID No. 49: 52%-62%; SEQ ID No. 51: 55%-73% and SEQ ID No. 53: 44%-80%.

The polynucleotides and polypeptides in the Sequence Listing and the identified homologous sequences may be stored in a computer system and have associated or linked with the sequences a function, such as that the polynucleotides and polypeptides are useful for modifying the environmental stress tolerance of a plant.

All references, publications, patents and other documents herein are incorporated by reference in their entirety for all purposes. Although the invention has been described with

reference to the embodiments and examples above, it should be understood that various modifications can be made without departing from the spirit of the invention.

What is claimed is:

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1. A transgenic plant with modified environmental stress tolerance, which plant comprises a recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-27, or a complementary nucleotide sequence thereof;

 (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);
 - (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-27, or a complementary nucleotide sequence thereof;
 - (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
 - (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
 - (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e);
 - (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide that modifies a plant's environmental stress tolerance;
 - (h) a nucleotide sequence having at least 30% sequence identity to a nucleotide sequence of any of (a)-(g);
 - (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g);
 - (j) a nucleotide sequence which encodes a polypeptide having at least 30% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27;
- 25 (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27; and (l) a nucleotide sequence which encodes a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-27.
- The transgenic plant of claim 1, further comprising a constitutive, inducible, or tissueactive promoter operably linked to said nucleotide sequence.
 - 3. The transgenic plant of claim 1, wherein the plant is selected from the group consisting of: soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf,

banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, and vegetable brassicas.

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- 4. An isolated or recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:
 - (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-27, or a complementary nucleotide sequence thereof;
- (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);
 - (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-27, or a complementary nucleotide sequence thereof;
 - (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
 - (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
 - (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e);
 - (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide that modifies a plant's environmental stress tolerance;
 - (h) a nucleotide sequence having at least 30% sequence identity to a nucleotide sequence of any of (a)-(g);
 - (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g);
 - (j) a nucleotide sequence which encodes a polypeptide having at least 30% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27;
 - (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27; and
- 30 (l) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-27.

5. The isolated or recombinant polynucleotide of claim 4, further comprising a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence.

- A cloning or expression vector comprising the isolated or recombinant polynucleotide of
 claim 4.
 - 7. A cell comprising the cloning or expression vector of claim 6.
 - 8. A transgenic plant comprising the isolated or recombinant polynucleotide of claim 4.

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- 9. A composition produced by one or more of:
 - (a) incubating one or more polynucleotide of claim 4 with a nuclease;
 - (b) incubating one or more polynucleotide of claim 4 with a restriction enzyme;
 - (c) incubating one or more polynucleotide of claim 4 with a polymerase;
 - (d) incubating one or more polynucleotide of claim 4 with a polymerase and a primer;
 - (e) incubating one or more polynucleotide of claim 4 with a cloning vector, or
 - (f) incubating one or more polynucleotide of claim 4 with a cell.
- 10. A composition comprising two or more different polynucleotides of claim 4.

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- 11. An isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide of claim 4.
- 12. A plant ectopically expressing an isolated polypeptide of claim 11.

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- 13. A method for producing a plant having a modified environmental stress tolerance, the method comprising altering the expression of the isolated or recombinant polynucleotide of claim 4 or the expression levels or activity of a polypeptide of claim 11 in a plant, thereby producing a modified plant, and selecting the modified plant for improved environmental stress tolerance thereby providing the modified plant with a modified environmental stress tolerance.
- 14. The method of claim 13, wherein the polynucleotide is a polynucleotide of claim 4.

15. A method of identifying a factor that is modulated by or interacts with a polypeptide encoded by a polynucleotide of claim 4, the method comprising:

- (a) expressing a polypeptide encoded by the polynucleotide in a plant; and
- (b) identifying at least one factor that is modulated by or interacts with the polypeptide.

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- 16. The method of claim 15, wherein the identifying is performed by detecting binding by the polypeptide to a promoter sequence, or detecting interactions between an additional protein and the polypeptide in a yeast two hybrid system.
- 10 17. The method of claim 15, wherein the identifying is performed by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.
 - 18. A method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest, the method comprising:
 - (a) placing the molecule in contact with a plant comprising the polynucleotide or polypeptide encoded by the polynucleotide of claim 4; and,
 - (b) monitoring one or more of:
 - (i) expression level of the polynucleotide in the plant;
 - (ii) expression level of the polypeptide in the plant;
 - (iii) modulation of an activity of the polypeptide in the plant; or
 - (iv) modulation of an activity of the polynucleotide in the plant.

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- 19. An integrated system, computer or computer readable medium comprising one or more character strings corresponding to a polynucleotide of claim 4, or to a polypeptide encoded by the polynucleotide.
 - 20. The integrated system, computer or computer readable medium of claim 19, further comprising a link between said one or more sequence strings to a modified plant environmental stress tolerance phenotype.

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- 21. A method of identifying a sequence similar or homologous to one or more polynucleotides of claim 4, or one or more polypeptides encoded by the polynucleotides, the method comprising:
 - (a) providing a sequence database; and,

(b) querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.

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- 22. The method of claim 21, wherein the querying comprises aligning one or more of the target sequences with one or more of the one or more sequence members in the sequence database.
- 10 23. The method of claim 21, wherein the querying comprises identifying one or more of the one or more sequence members of the database that meet a user-selected identity criteria with one or more of the target sequences.
 - 24. The method of claim 21, further comprising linking the one or more of the
- polynucleotides of claim 4, or encoded polypeptides, to a modified plant environmental stress tolerance phenotype.
 - 25. A plant comprising altered expression levels of an isolated or recombinant polynucleotide of claim 4.

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- 26. A plant comprising altered expression levels or the activity of an isolated or recombinant polypeptide of claim 11.
- 27. A plant lacking a nucleotide sequence encoding a polypeptide of claim 11.

Figure 1

| SEQ ID No. | GID | cDNA or protein | conserved domain |
|------------|------|-----------------|----------------------------|
| 1 | G22 | cDNA | |
| 2 | G22 | protein | 89-157 |
| 3 | G188 | cDNA | |
| 4 | G188 | protein | 175-222 |
| 5 | G225 | cDNA | |
| 6 | G225 | protein | 39-76 |
| 7 | G226 | cDNA | |
| 8 | G226 | protein | 28-78 |
| 9 | G256 | cDNA | |
| 10 | G256 | protein | 13-115 |
| 11 | G419 | cDNA | |
| 12 | G419 | protein | 392-452 |
| 13 | G464 | cDNA | |
| 14 | G464 | protein | 7-15,70-80,125-158,183-219 |
| 15 | G482 | cDNA . | |
| 16 | G482 | protein | 25-116 |
| 17. | G502 | cDNA | |
| 18 | G502 | protein | 10-155 |
| 19 | G526 | cDNA | |
| 20 | G526 | protein | 21-149 |
| 21 | G545 | cDNA | |
| 22 | G545 | protein | 82-102, 136-154 |
| 23 | G561 | cDNA | |
| 24 | G561 | protein | 248-308 |
| 25 | G664 | cDNA | |
| 26 | G664 | protein | 13-116 |
| 27 | G682 | cDNA | |
| 28 | G682 | protein | 22-53 |
| 29 | G911 | cDNA | |
| 30 | G911 | protein | 86-129 |
| 31 | G964 | cDNA | |
| 32 | G964 | protein | 126-186 |
| 33 | G394 | cDNA | |
| 34 | G394 | protein | 121-182 |
| 35 | G489 | cDNA | |
| 36 | G489 | protein | 57-156 |

Figure 2

| SEQ ID No. | IGID | homolog | cDNA or protein | conserved domain |
|------------|-------|-----------------|-----------------|--------------------------------|
| 37 | G463 | homolog of G464 | CDNA | |
| 38 | G463 | homolog of G464 | protein | 14-23, 77-88, 130-146, 194-227 |
| 39 | G767 | homolog of G502 | cDNA | |
| 40 | G767 | homolog of G502 | protein | 8-158 |
| 41 | G765 | homolog of G526 | cDNA | |
| 42 | G765 | homolog of G526 | protein | 23-167 |
| 43 | G197 | homolog of G664 | cDNA | |
| 44 | G197 | homolog of G664 | protein | 14-119 |
| 45 | G255 | homolog of G664 | cDNA | · |
| 46 | G255 | homolog of G664 | protein | 14-115 |
| 47 | G1113 | homolog of G911 | cDNA | |
| 48 | G1113 | homolog of G911 | protein | 85-128 |
| 49 | G398 | homolog of G964 | cDNA | |
| 50 | G398 | homolog of G964 | protein | 128-191 |
| 51 | G395 | homolog of G394 | cDNA | |
| 52 | G395 | homolog of G394 | protein | 72-135 |
| 53 | G393 | homolog of G394 | cDNA | |
| 54 | G393 | homolog of G394 | protein | 106-169 |

Figure 3A

| SEQ ID No. | GID | Genbank NID | P-value | Species |
|------------|--------------------|-------------|-----------|---------------------------------------|
| 1 | G22 | 790359 | 1.00E-45 | Nicotiana tabacum |
| 1 | G22 | 3342210 | 6.60E-45 | Lycopersicon esculentum |
| 1 | G22 | 6654776 | 1.60E-44 | Medicago truncatula |
| 1 | G22 | 8809570 | 5.80E-44 | Nicotiana sylvestris |
| 1 | G22 | 7627061 | 2.40E-39 | Gossypium arboreum |
| 1 | G22 | 7324479 | 9.50E-36 | Lycopersicon pennellii |
| 1 | G22 | 8980312 | 4.30E-31 | Catharanthus roseus |
| 1 | G22 | 7528275 | 1.20E-30 | Mesembryanthemum crystallinum |
| 1 | G22 | 6478844 | 4.60E-28 | Matricaria chamomilla |
| 1 | G22 | 6847348 | 5.90E-26 | Glycine max |
| 3 | G188 | 7779802 | 5.20E-36 | Lotus japonicus |
| 3. | G188 | 7284340 | 2.10E-34 | Glycine max |
| 3 | G188 | 9361307 | 1.20E-27 | Triticum aestivum |
| 3 | G188 | 7340336 | ·1.10E-22 | Oryza sativa |
| 3 | G188 | 6529152 | 3.60E-22 | Lycopersicon esculentum |
| 3 | G188 | 8748477 | 7.70E-21 | Medicago truncatula |
| 3 | G188 | 5456433 | 7.10E-14 | Zea mays |
| 3 | G188 | 9302479 | 1.60E-12 | Sorghum bicolor |
| 3 | G188 | 6696287 | 4.10E-12 | Pinus taeda |
| 3 | G188 | 562242 | 9.00E-12 | Brassica rapa |
| 5 | G225 | 4396287 | 4.40E-16 | Glycine max |
| 5 | G225 | 309571 | 0.00029 | Zea mays |
| 5 | G225 | 3857004 | 0.001 | Populus tremula x Populus tremuloides |
| 5 | G225 | 9410205 | 0.019 | Triticum aestivum |
| 5 | G225 | 9426190 | 0.025 | Triticum turgidum subsp. durum |
| 5 | G225 | 8382118 | 0.046 | Gossypium arboreum |
| 5 | G225 | 6782756 | 0.27 | Oryza sativa |
| 5 | G225 | 7721017 | 0.4 | Lotus japonicus |
| 5 | G225 | 6020136 | 0.47 | Pinus taeda |
| 5 | ·G225 | 2921331 | 0.48 | Gossypium hirsutum |
| 7 | G226 | 4396287 | 5.10E-15 | Glycine max |
| 7 | G226 | 9410205 | 1.50E-05 | Triticum aestivum |
| 7 | G226 | 3857004 | 0.11 | Populus tremula x Populus tremuloides |
| 7 | G226 | 2428139 | 0.35 | Oryza sativa |
| 9 | G256 | 1430847 | 1.30E-72 | Lycopersicon esculentum |
| 9 | G256 | 9252441 | 1.20E-65 | Solanum tuberosum |
| 9 | G256 | 8380712 | 2.20E-58 | Gossypium arboreum |
| 9 | G256 | 8172976 | 1.60E-54 | Medicago truncatula |
| 9 | G256 | 9205295 | 1.30E-44 | Glycine max |
| 9 | G256 | 20562 | 6.40E-40 | Petunia x hybrida |
| 9 | G256 | 4886263 | 4.40E-37 | Antirrhinum majus |
| 9 | G256 | 6552360 | 5.00E-36 | Nicotiana tabacum |
| 9 | G256 | 2312003 | 1.20E-35 | Oryza sativa |
| 9 | G256 | 5268628 | 5.20E-35 | Zea mays |
| 11 | G419 | 7239156 | 2.60E-59 | Malus x domestica |
| 11 | G419 | 5278451 | 9.00E-58 | Lycopersicon esculentum |
| 11 | G419 | 9205496 | 1.30E-55 | Glycine max |
| 11 | G419 | 7628137 | 9.30E-51 | Gossypium arboreum |
| 11 | G419 | 6069643 | 9.50E-51 | Oryza sativa |
| 11 | G419 | 7562931 | 9.80E-45 | Medicago truncatula |
| 11 | G419 | 7322293 | 2.30E-37 | Lycopersicon hirsutum |
| 11 | G419 | 8404716 | 1.10E-29 | Hordeum vulgare |
| 11 | G419 | 7217755 | 1.40E-29 | Sorghum bicolor |
| | _ · · - | | | |

Figure 3B

| rigule 35 | | | | Canadian |
|------------|------|-------------|----------|-------------------------------|
| SEQ ID No. | GID | Genbank NID | | Species |
| 11 | G419 | 9428023 | 4.60E-28 | Triticum aestivum |
| 13 | G464 | 6527230 | 3.60E-31 | Lycopersicon esculentum |
| 13 | G464 | 9305572 | 1.10E-22 | Sorghum bicolor |
| 13 | G464 | 6604917 | 6.70E-22 | Medicago truncatula |
| 13 | G464 | 5058123 | 2.30E-21 | Glycine max |
| 13 | G464 | 3760881 | 1.20E-19 | Oryza sativa |
| 13 | G464 | 5044476 | 1.20E-17 | Gossypium hirsutum |
| 13 | G464 | 9412603 | 6.40E-15 | Triticum aestivum |
| 13 | G464 | 7777277 | 3.20E-13 | Lotus japonicus |
| 13 | G464 | 9410371 | 1.70E-11 | Hordeum vulgare |
| 13 | G464 | 7624108 | 2.10E-10 | Gossypium arboreum |
| 15 | G482 | 7691987 | 5.50E-50 | Glycine max |
| 15 | G482 | 7781090 | 1.30E-48 | Lotus japonicus |
| 15 | G482 | 7409616 | 1.10E-47 | Lycopersicon esculentum |
| 15 | G482 | 9416562 | 4.40E-46 | Triticum aestivum |
| 15 | G482 | 22379 | 2.30E-44 | Zea mays |
| 15 | G482 | 7501372 | 7.70E-44 | Gossypium arboreum |
| 15 | G482 | 7765436 | 8.40E-42 | Medicago truncatula |
| 15 | G482 | 5044464 | 1.20E-40 | Gossypium hirsutum |
| 15 | G482 | 9441376 | 9.20E-40 | Chlamydomonas reinhardtii |
| 15 | G482 | 8071558 | 3.50E-39 | Solanum tuberosum |
| 17 | G502 | 6730941 | 1.60E-91 | Oryza sativa |
| 17 | G502 | 7765679 | 1.60E-82 | Medicago truncatula |
| 17 | G502 | 7502501 | 7.30E-80 | Gossypium arboreum |
| 17 | G502 | 5510359 | 8.30E-77 | Glycine max |
| | G502 | 5601137 | 8.70E-76 | Lycopersicon esculentum |
| 17 | G502 | 9302206 | 1.40E-73 | Sorghum bicolor |
| 17 | | 4089948 | 3.40E-50 | Brassica napus |
| 17 | G502 | 8329134 | 7.90E-49 | Mesembryanthemum crystallinum |
| 17 | G502 | | 8.60E-49 | Lotus japonicus |
| 17 | G502 | 7723564 | 1.80E-48 | Triticum sp. |
| 17 | G502 | 4218534 | 3.40E-61 | Gossypiüm hirsutum |
| 19 | G526 | 5049217 | 1.50E-55 | Petunia x hybrida |
| 19 | G526 | 6066594 | 1.50E-54 | Lycopersicon esculentum |
| 19 | G526 | 4384535 | | Glycine max |
| 19 | G526 | 6454868 | 6.60E-54 | Oryza sativa |
| 19 | G526 | 4977542 | 4.70E-52 | Zea mays |
| 19 | G526 | 5343151 | 7.00E-51 | Triticum aestivum |
| 19 | G526 | 9361647 | 5.10E-50 | Medicago truncatula |
| 19 | G526 | 6799764 | 4.30E-48 | Hordeum vulgare |
| 19 | G526 | 8708684 | 1.80E-47 | Triticum sp. |
| 19 | G526 | 4218536 | 3.60E-47 | |
| 21 | G545 | 4666359 | 8.30E-55 | |
| 21 | G545 | 7228328 | 3.70E-52 | |
| 21 | G545 | 1763062 | 1.30E-51 | |
| 21 | G545 | 7206360 | 3.10E-44 | |
| 21 | G545 | 7626808 | 9.60E-40 | |
| 21 | G545 | 439492 | 3.90E-39 | |
| 21 | G545 | 4382658 | 1.70E-38 | |
| 21 | G545 | 8486215 | 8.70E-38 | |
| 21 | G545 | | 6.80E-37 | |
| 21 | G545 | | 1.10E-33 | |
| 23 | G561 | 2995461 | 5.60E-86 | |
| 23 | G561 | 633153 | 6.50E-83 | Brassica napus |

Figure 3C

| SEQ ID No. | GID | Genbank NID | P-value | Species |
|------------|------|-------------|----------|---------------------------------------|
| 23 | G561 | 1033058 | 5.90E-65 | Raphanus sativus |
| 23 | G561 | 2815304 | 2.10E-35 | Spinacia oleracea |
| 23 | G561 | 1498300 | 1.60E-34 | Petroselinum crispum |
| | | | 8.10E-32 | |
| 23 | G561 | 169958 | | Glycine max Catharanthus roseus |
| 23 | G561 | 5381310 | 2.20E-30 | |
| 23 | G561 | 1155053 | 9.70E-28 | Phaseolus vulgaris |
| 23 | G561 | 728627 | 1.90E-27 | Nicotiana tabacum |
| 23 | G561 | 7565950 | 1.40E-21 | Medicago truncatula |
| 25 | G664 | 1167483 | 4.90E-81 | Lycopersicon esculentum |
| 25 | G664 | 7765706 | 6.30E-69 | Medicago truncatula |
| 25 | G664 | 19052 | 9.30E-68 | Hordeum vulgare |
| 25 | G664 | 7626566 | 4.00E-67 | Gossypium arboreum |
| 25 | G664 | 5050757 | 2.60E-66 | Gossypium hirsutum |
| 25 | G664 | 6850206 | 6.90E-66 | Oryza sativa |
| 25 | G664 | 6667606 | 2.20E-63 | Glycine max |
| 25 | G664 | 517492 | 9.30E-62 | Zea mays |
| 25 | G664 | 9302672 | 1.50E-59 | Sorghum bicolor |
| 25 | G664 | 5860031 | 9.20E-58 | Pinus taeda |
| 27 | G682 | 309571 | 4.40E-08 | Zea mays |
| 27 | G682 | 4396287 | 1.10E-05 | Glycine max |
| 27 | G682 | 3857004 | 0.00051 | Populus tremula x Populus tremuloides |
| 27 | G682 | 9410205 | 0.00085 | Triticum aestivum |
| 27 | G682 | 8382118 | 0.0079 | Gossypium arboreum |
| 27 | G682 | 2428139 | 0.017 | Oryza sativa |
| 27 | G682 | 7339148 | 0.13 | Lycopersicon esculentum |
| 27 | G682 | 9302672 | 0.32 | Sorghum bicolor |
| 27 | G682 | 5048991 | 0.39 | Gossypium hirsutum |
| 27 | G682 | 6555777 | 0.46 | Pinus taeda |
| 29 | G911 | 4090113 | 6.10E-51 | Brassica napus |
| 29 . | G911 | 5893315 | 7.70E-25 | Lycopersicon esculentum |
| 29 | G911 | 5048452 | 3.10E-23 | Gossypium hirsutum |
| 29 | G911 | 9440241 | 1.90E-21 | Glycine max |
| 29 | G911 | 6917169 | 1.80E-11 | Lycopersicon pennellii |
| 29 | G911 | 9297970 | 3.20E-11 | Sorghum bicolor |
| 29 | G911 | 7137594 | 4.90E-11 | Zea mays |
| 29 | G911 | 9278447 | 4.60E-10 | Lotus japonicus |
| 29 | G911 | 7560271 | 7.20E-10 | Medicago truncatula |
| 29 | G911 | 5043346 | 4.50E-09 | Sorghum halepense |
| 31 | G964 | 7624806 | 3.30E-72 | Gossypium arboreum |
| 31 | G964 | 1234899 | 9.10E-66 | Glycine max |
| 31 | G964 | 1149534 | 1.50E-61 | Pimpinella brachycarpa |
| 31 | G964 | 8919872 | 3.40E-51 | Capsella rubella |
| 31 | G964 | 992597 | 6.70E-51 | Lycopersicon esculentum |
| 31 | G964 | 1235564 | 1.50E-38 | Oryza sativa |
| 31 | G964 | 6605613 | 3.00E-32 | Medicago truncatula |
| 31 | G964 | 1032371 | 4.50E-28 | Helianthus annuus |
| 31 | G964 | 3868846 | 2.80E-25 | Ceratopteris richardii |
| 31 | G964 | 8088109 | 6.40E-22 | Sorghum bicolor |
| 33 | G394 | 8670502 | 7.90E-59 | Glycine max |
| 33 | G394 | 3171738 | 2.00E-54 | Craterostigma plantagineum |
| 33 | G394 | 1032371 | 1.10E-50 | Helianthus annuus |
| 33 | G394 | 7624806 | 4.30E-47 | Gossypium arboreum |
| | | | | |
| 33 | G394 | 1160483 | 2.10E-46 | Pimpinella brachycarpa |

Figure 3D

| SEQ ID No. | GID | Genbank NID | | Species |
|------------|------|---------------------------------------|----------|-------------------------------|
| 33 | G394 | 3868846 | 4.20E-45 | Ceratopteris richardii |
| 33 | G394 | 992597 | 1.10E-44 | Lycopersicon esculentum |
| 33 | G394 | 7558511 | 1.50E-44 | Medicago truncatula |
| 33 | G394 | 8099247 | 6.20E-43 | Oryza sativa |
| 33 | G394 | 8919872 | 1.20E-40 | Capsella rubella |
| 35 | G489 | 6534956 | 4.40E-62 | Lycopersicon esculentum |
| 35 | G489 | 9055852 | 2.60E-60 | Medicago truncatula |
| 35 | G489 | 8382393 | 6.20E-51 | Gossypium arboreum |
| 35 | G489 | 8789169 | 2.10E-50 | Citrus x paradisi |
| 35 | G489 | 9252957 | 1.50E-47 | Solanum tuberosum |
| 35 | G489 | 6918056 | 4.70E-47 | Lycopersicon pennellii |
| 35 | G489 | 7590809 | 1.00E-46 | Glycine max |
| 35 | G489 | 5257255 | 8.60E-43 | Oryza sativa |
| 35 | G489 | 4152190 | 3.20E-41 | Zea mays |
| 35 | G489 | 6069260 | 2.10E-39 | Ceratodon purpureus |
| 37 | G463 | 6527230 | 4.90E-36 | Lycopersicon esculentum |
| 37 | G463 | 9305572 | 5.50E-36 | Sorghum bicolor |
| 37 | G463 | 3760881 | 1.20E-31 | Oryza sativa |
| 37 | G463 | 6604917 | 1.30E-23 | Medicago truncatula |
| | G463 | 5058123 | 2.50E-21 | Glycine max |
| 37 | G463 | 5044476 | 1.10E-19 | Gossypium hirsutum |
| 37 | | 9412603 | 1.70E-17 | Triticum aestivum |
| 37 | G463 | 9419394 | 6.00E-17 | Hordeum vulgare |
| 37 | G463 | · · · · · · · · · · · · · · · · · · · | 6.20E-17 | Gossypium arboreum |
| 37 | G463 | 7624108 | 3.20E-16 | Nicotiana tabacum |
| 37 | G463 | 8547152 | 2.80E-76 | Glycine max |
| 39 | G767 | 5510359 | 4.20E-74 | Medicago truncatula |
| 39 | G767 | 7643155 | 1.10E-72 | Lycopersicon esculentum |
| 39 | G767 | 6977319 | 4.20E-68 | Oryza sativa |
| 39 | G767 | 6730939 7502501 | 2.00E-67 | Gossypium arboreum |
| 39 | G767 | 9302206 | 3.10E-65 | Sorghum bicolor |
| 39 | G767 | 4218534 | 4.30E-51 | Triticum sp. |
| 39 | G767 | 6732157 | 4.30E-51 | Triticum monococcum |
| 39 | G767 | | 6.90E-47 | Triticum aestivum |
| 39 | G767 | 9412602 | 1.30E-46 | Mesembryanthemum crystallinum |
| 39 | G767 | 8329134 4384535 | 3.10E-56 | Lycopersicon esculentum |
| 41 | G765 | | 8.50E-56 | Glycine max |
| 41 | G765 | 6454868 | 4.30E-53 | Petunia x hybrida |
| 41 | G765 | 1279639 | 2.00E-51 | Oryza sativa |
| 41 | G765 | 4977542 4218536 | 2.00E-50 | Triticum sp. |
| 41 | G765 | | 2.00E-50 | Triticum monococcum |
| 41 | G765 | 6732159 | 6.90E-50 | Gossypium hirsutum |
| 41 | G765 | 5049217 | 4.50E-49 | Triticum aestivum |
| 41 | G765 | 9361647 | 2.90E-48 | Sorghum bicolor |
| 41 | G765 | 9296257 | 4.30E-46 | Hordeum vulgare |
| 41 | G765 | 8708684 | 2.70E-76 | Lycopersicon esculentum |
| 43 | G197 | 1167483 | 2.40E-73 | Gossypium arboreum |
| 43 | G197 | 7626566 | 1.50E-63 | Medicago truncatula |
| 43 | G197 | 7765706 | | Hordeum vulgare |
| 43 | G197 | 19052 | 8.90E-63 | Gossypium hirsutum |
| 43 | G197 | 5050757 | 1.60E-62 | Oryza sativa |
| 43 | G197 | 6850206 | 1.10E-61 | Glycine max |
| 43 | G197 | 6667606 | 1.70E-61 | |
| 43 | G197 | 517492 | 7.60E-59 | Zea mays |

Figure 3E

| SEQ ID No. | GID | Genbank NID | P-value | Species |
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| 43 | G197 | 5860031 | 3.90E-57 | Pinus taeda |
| 43 | G197 | 9302672 | 3.80E-55 | Sorghum bicolor |
| 45 | G255 | 1167483 | 6.40E-75 | Lycopersicon esculentum |
| 45 | G255 | 7626566 | 6.40E-71 | Gossypium arboreum |
| 45 | G255 | 19050 | 2.80E-65 | Hordeum vulgare |
| 45 | G255 | 5050757 | 3.70E-63 | Gossypium hirsutum |
| 45 | G255 | 7590249 | 4.10E-62 | Glycine max |
| 45 | G255 | 7765706 | 4.40E-62 | Medicago truncatula |
| 45 | G255 | 6850206 | 1.10E-61 | Oryza sativa |
| 45 | G255 | 517492 | 3.50E-59 | Zea mays |
| 45 | G255 | 9302672 | 1.60E-56 | Sorghum bicolor |
| 45 | G255 | 7721017 | 2.60E-55 | Lotus japonicus |
| 47 | G1113 | .4090113 | 2.30E-36 | Brassica napus |
| 47 | G1113 | 5048452 | 6.80E-12 | Gossypium hirsutum |
| 47 | G1113 | 5893315 | 9.50E-11 | Lycopersicon esculentum |
| 47 | G1113 | 9440241 | 7.70E-09 | Glycine max |
| 49 | G398 | 7624806 | 2.80E-67 | Gossyplum arboreum |
| 49 | G398 | 1234899 | 6.90E-64 | Glycine max |
| 49 | G398 | 1149534 | 6.20E-63 | Pimpinella brachycarpa |
| 49 | G398 | 8919872 | 2.60E-47 | Capsella rubella |
| 49 | G398 | 992597 | 1.10E-39 | Lycopersicon esculentum |
| 49 | G398 | 1235564 | 7.70E-39 | Oryza sativa |
| 49 | G398 | 6605613 | 1.70E-33 | Medicago truncatula |
| 49 | G398 | 8088109 | 3.60E-33 | Sorghum bicolor |
| 49 | G398 | 3868846 | 1.60E-32 | Ceratopteris richardii |
| 49 | G398 | 3171738 | 1.00E-27 | Craterostigma plantagineum |
| 51 | G395 | 992597 | 5.30E-51 | Lycopersicon esculentum |
| 51 | G395 | 7624806 | 2.00E-50 | Gossypium arboreum |
| 51 | G395 | 1234899 | 1.50E-49 | Glycine max |
| 51 | G395 | 1165131 | 1.90E-48 | Pimpinella brachycarpa |
| 51 | G395 | 3868846 | 3.40E-47 | Ceratopteris richardii |
| 51 | G395 | 7415619 | 1.30E-41 | Physcomitrella patens |
| 51 | G395 | 8919872 | 7.40E-41 | Capsella rubella |
| 51 | G395 | 1235564 | 2.70E-38 | Oryza sativa |
| 51 | G395 | 8088109 | 2.30E-33 | Sorghum bicolor |
| 51 | G395 | 1032371 | 3.30E-31 | Helianthus annuus |
| 53 | G393 | 8670502 | 3.60E-55 | Glycine max |
| 53 | G393 | 9199975 | 7.60E-46 | Medicago truncatula |
| 53 | G393 | 3868846 | 9.60E-37 | Ceratopteris richardii |
| 53 | G393 | 8919872 | 2.50E-35 | Capsella rubella |
| 53 | G393 | 7624806 | 1.30E-34 | Gossypium arboreum |
| 53 | G393 | 7415619 | 1.00E-33 | Physcomitrella patens |
| 53 | G393 | 5897000 | 5.50E-33 | Lycopersicon esculentum |
| 53 | G393 | 1235564 | 4.00E-32 | Oryza sativa |
| 53 | G393 | 1165131 | 6.40E-32 | Pimpinella brachycarpa |
| 53 | G393 | 3171738 | 1.50E-31 | Craterostigma plantagineum |

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MBI16 Sequence Listing.ST25 SEQUENCE LISTING

| <110> Pineda, Omaira Yu, Guo-Liang Creelman, Robert Riechmann, Jose Luis Heard, Jacqueline Ratcliffe, Oliver Reuber, Lynne Keddie, James | |
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| aga Arg 140 | gga Gly | tcg Ser | aaa Lys | gct Ala | aag Lys 145 | ctg Leu | aat Asn | ttt Phe | ccg Pro | cat His 150 | ttg Leu | att Ile | ggt Gly | tct Ser | tgt Cys 155 | 545 |
| aag Lys | tat Tyr | gag Glu | ccg Pro | gtt Val 160 | agg Arg | att Ile | agg Arg | cct Pro | cgc Arg 165 | cgt Arg | cgc Arg | tcg Ser | ccg Pro | gaa Glu 170 | ccg Pro | 593 |
| tca Ser | gtc Val | tcc Ser | gat Asp 175 | cag Gln | tta Leu | acg Thr | tcg Ser | gag Glu 180 | cag Gln | aag Lys | agg Arg | gaa Glu | agc Ser 185 | cac His | gtg Val | 641 |
| gat Asp | gac Asp | ggc Gly 190 | gag Glu | tct Ser | agt Ser | ttg Leu | gtt Val 195 | gta Val | ccg Pro | gag Glu | ttg Leu | gat Asp 200 | ttc Phe | acg Thr | gtg Val | 689 |
| gat Asp | cag Gln 205 | ttt Phe | tac Tyr | ttc Phe | gat Asp | ggt Gly 210 | agt Ser | tta Leu | tta Leu | atg Met | gac Asp 215 | caa Gln | tca Ser | gaa Glu | tgt Cys | 737 |
| | | tct Ser | | | | | taa | ttag | gttt | aa g | gatta | agca | aa aa | atttg | gtcca | 791 |
| acga | agttt | tg c | tgta | atgaa | aa ta | atcta | atcga | a tga | actca | aca | ggtt | ttg | atc a | atgat | catat | 851 |
| gtaa | atgto | gat g | gaaa | attaa | aa ta | attga | acgti | tgt: | tttt | ttg | ttgt | aaaa | aaa a | aaaa | aaaaa | 911 |
| aa | | | | | | | | | | | | | | | | 913 |
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| Phe | Arg | Asn | Pro 20 | Ser | Phe | Ser | Asn | Val 25 | Ile | Leu | Asn | Asp | Asn 30 | Trp | Ser | |
| Asp | Leu | Pro 35 | Leu | Ser | Val | Asp | Asp 40 | Ser | Gln | Авр | Met | Ala 45 | Ile | Tyr | Asn | |
| Thr | Leu 50 | Arg | 'Asp | Ala | Val | Ser 55 | Ser | Gly | Trp | Thr | Pro 60 | Ser | Val | Pro | Pro | |
| Val 65 | Thr | Ser | Pro | Ala | Glu 70 | Glu | Asn | Lys | Pro | Pro 75 | Ala | Thr | Lys | Ala | Ser 80 | • |
| Gly | Ser | His | Ala | Pro 85 | Arg | Gln | Lys | Gly | Met 90 | Gln | Tyr | Arg | Gly | Val 95 | Arg | |
| Arg | Arg | Pro | Trp 100 | Gly | Lys | Phe | Ala | Ala 105 | Glu | Ile | Arg | Asp | Pro 110 | Lys | Lys | |
| Asn | Gly | Ala 115 | Arg | Val | Trp | Leu | Gly 120 | Thr | Tyr | Glu | Thr | Pro 125 | Glu | Asp | Ala | |
| Ala | | | | | | | | | | | | | | | | |

Page 2

| Lys 145 | Leu | Asn | Phe | Pro | His 150 | Leu | Ile | Gly | Ser | Cys 155 | Lys | Tyr | Glu | Pro | Val 160 | |
|--|--|--|---|--|---|--|--|---|--|---|---|---|--|---|--|---------------------------------|
| Arg | Ile | Arg | Pro | Arg 165 | Arg | Arg | Ser | Pro | Glu 170 | Pro | Ser | Val | Ser | Asp 175 | Gln | |
| Leu | Thr | Ser | Glu 180 | Gln | Lys | Arg | Glu | Ser 185 | His | Val | Asp | Asp | Gly 190 | Glu | Ser | |
| Ser | Leu | Val 195 | Val | Pro | Glu | Leu | Asp 200 | Phe | Thr | Val | Asp | Gln 205 | Phe | Tyr | Phe | |
| qaA | Gly 210 | Ser | Leu | Leu | Met | Asp 215 | Gln | Ser | Glu | Суз | Ser 220 | Туг | Ser | Asp | Asn | |
| Arg 225 | Ile | | | | | | | | | | | | | | | |
| <21 <21 <21 <21 | l> : 2> I | 3 1195 ONA Arab: | idop | sis 1 | thali | lana | | | | | | | | | | |
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| | | | | | | | | | | | | | 1-16 | | er ser | |
| | | | | | | | | | | | | | 1 | 3C 3C | er ser | |
| | | | gat Asp | | | | | | | | | | 1 tct | tct | gtt | 106 |
| Glu | Asp 5 acc | Trp | | Leu | Phe tgt | Ala 10 gct | Val ggt | Val cat | Arg | Ser | Cys 15 gac | Ser | tct Ser | tct Ser | gtt Val tgt | 106 154 |
| tcc Ser 20 | Asp 5 acc Thr | acc Thr | Asp | tct Ser | tgt Cys 25 | Ala 10 gct Ala cct | Val ggt Gly cct | Val cat His | Arg gaa Glu cct | gac Asp 30 | Cys 15 gac Asp | ser ata Ile caa | tct Ser gga Gly | tct Ser aac Asn | gtt Val tgt Cys 35 | |
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| tcc Ser 20 aaa Lys tct Ser act Thr | Asp 5 acc Thr caa Gln tgc Cys act Thr | Trp acc Thr caa Gln aac Asn act Thr 70 | Asp aat Asn caa Gln gag Glu 55 act | tct Ser gat Asp 40 tta Leu act Thr | Phe tgt Cys 25 cct Pro caa Gln act Thr | Ala 10 gct Ala cct Pro gat Asp tgg Trp ccc | yal ggt Gly cct Pro tct Ser tct Ser 75 | val cat His cct Pro tgc Cys 60 cct Pro | gaa Glu cct Pro 45 aaa Lys cct Pro | gac Asp 30 ctg Leu cca Pro cct | Cys 15 gac Asp ttt Phe ttt Phe cta Leu | ser ata Ile caa Gln tta Leu ctt Leu 80 caa | tct Ser gga Gly gct Ala ccc Pro 65 cct Pro | tct Ser aac Asn tct Ser 50 gtt Val cct Pro | gtt Val tgt Cys 35 tct Ser act Thr | 154 202 250 |
| tcc Ser 20 aaaa Lys tct Ser act Thr aaaa Lys | Asp 5 acc Thr caa Gln tgc Cys act Thr gcc Ala 85 | Trp acc Thr caa Gln aac Asn act Thr 70 tca Ser | Āsp aat Asn caa Gln gag Glu 55 act Thr | tct Ser gat Asp 40 tta Leu act Thr | tgt Cys 25 cct Pro caa Gln act Thr | Ala 10 gct Ala cct Pro gat Asp tgg Trp ccc Pro 90 caa | ggt Gly cct Pro tct Ser tct Ser tct Asn | val cat His cct Pro tgc Cys 60 cct Pro | gaa Glu cct Pro 45 aaa Lys cct Pro tta Leu cct | gac Asp 30 ctg Leu cca Pro cta Leu ctt | Cys 15 gac Asp ttt Phe ttt Phe cta Leu aaa Lys 95 agt | ata Ile caa Gln tta Leu ctt Leu ctt Leu gtt | tct Ser gga Gly gct Ala ccc Pro 65 cct Pro | tct Ser aac Asn tct Ser 50 gtt Val cct Pro | gtt Val tgt Cys 35 tct Ser act Thr | 154 202 250 298 |
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MBI16 Sequence Listing.ST25
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Pro Pro Pro Lys Ala Ser Ser Pro Ser Pro Asn Ile Leu Leu Lys Gln 85 90 95

Glu Gln Val Leu Leu Glu Ser Gln Asp Gln Lys Pro Pro Leu Ser Val

Arg Val Phe Pro Pro Ser Thr Ser Ser Ser Val Phe Val Phe Arg Gly

Gln Arg Asp Gln Leu Leu Gln Gln Gln Ser Gln Pro Pro Leu Arg Ser 130 135 140

Arg Lys Arg Lys Asn Gln Gln Lys Arg Thr Ile Cys His Val Thr Gln 145 150 155 160

Glu Asn Leu Ser Ser Asp Leu Trp Ala Trp Arg Lys Tyr Gly Gln Lys 165 . 170 . 175

Pro Ile Lys Gly Ser Pro Tyr Pro Arg Asn Tyr Tyr Arg Cys Ser Ser 180 190

Ser Lys Gly Cys Leu Ala Arg Lys Gln Val Glu Arg Ser Asn Leu Asp 195 200 205

Pro Asn Ile Phe Ile Val Thr Tyr Thr Gly Glu His Thr His Pro Arg

Pro Thr His Arg Asn Ser Leu Ala Gly Ser Thr Arg Asn Lys Ser Gln 225 230 235 240

Pro Val Asn Pro Val Pro Lys Pro Asp Thr Ser Pro Leu Ser Asp Thr 245 250 255

Val Lys Glu Glu Ile His Leu Ser Pro Thr Thr Pro Leu Lys Gly Asn 265 270

Asp Asp Val Glu Glu Thr Asn Gly Asp Glu Asp Met Val Gly Gln Glu 275 280 285

Glu Glu Glu Asp Asp Asp Asp Val Asp Asp Leu Leu Ile Pro Asn 305 310 315 320

Page 5

PCT/US00/31458 WO 01/36598

MBI16 Sequence Listing.ST25

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MBI16 Sequence Listing.ST25

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85
90

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Page 7

MBI16 Sequence Listing.ST25

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85

90

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Thr His Leu Lys Lys Lys Leu Val Met Met Lys Phe Gln Asn Gly Ile

110 125 126

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| ctag | gctag | gt 1 | ttati | taati | t tt | ctt | ctt | t tgt | ctti | tct | ctai | gate | ett t | agti | acatt | 1430 |
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Gly Pro Gly Asn Trp Arg Ser Val Pro Ala Asn Thr Gly Leu Leu Arg Page 9

35

Cys Ser Lys Ser Cys Arg Leu Arg Trp Thr Asn Tyr Leu Arg Pro Gly 50 60

Ile Lys Arg Gly Asn Phe Thr Gln Pro Glu Glu Lys Met Ile Ile His 65 70 75 80

Leu Gln Ala Leu Leu Gly Asn Arg Trp Ala Ala Ile Ala Ser Tyr Leu 85 90 95

Pro Gln Arg Thr Asp Asn Asp Ile Lys Asn Tyr Trp Asn Thr His Leu 100 105 110

Lys Lys Leu Val Met Met Lys Phe Gln Asn Gly Ile Ile Asn Glu 115 120 125

Asn Lys Thr Asn Leu Ala Thr Asp Ile Ser Ser Cys Asn Asn Asn Asn 130 135 140

Asn Gly Cys Asn His Asn Lys Arg Thr Thr Asn Lys Gly Gln Trp Glu 145 150 150 160

Lys Lys Leu Gln Thr Asp Ile Asn Met Ala Lys Gln Ala Leu Phe Gln 165 170 175

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| gaa Glu | gag Glu 285 | ctt Leu | aaa Lys | aga Arg | aga Arg | tat Tyr 290 | gga Gly | cat His | tac Tyr | cga Arg | gag Glu 295 | caa Gln | atg Met | aga Arg | gtt Val | 1277 |
| gcg Ala 300 | gcg Ala | gca Ala | gcc Ala | ttt Phe | gaa Glu 305 | gcg Ala | gcg Ala | gtt Val | gga Gly | cta Leu 310 | gga Gly | G1 y | gca Ala | gag Glu | ata Ile 315 | 1325 |
| tac Tyr | act Thr | gcg Ala | tta Leu | gcg Ala 320 | tca Ser | agg Arg | gca Ala | atg Met | tca Ser 325 | aga Arg | cac His | ttt Phe | cgg Arg | tgt Cys 330 | tta Leu | 1373 |
| aaa Lys | gac Asp | gga Gly | ctt Leu 335 | gtg Val | gga Gly | cag Gln | att Ile | caa Gln 340 | gca Ala | aca Thr | agt Ser | caa Gln | gct Ala 345 | ttg Leu | gga Gly | 1421 |
| gag Glu | aga Arg | gaa Glu 350 | gag Glu | gat Asp | aat Asn | cgt Arg | gcg Ala 355 | gtt Val | tct Ser | att Ile | gca Ala | gca Ala 360 | cgt Arg | gga Gly | gaa Glu | 1469 |
| act Thr | cca Pro 365 | cgg Arg | ttg Leu | aga Arg | ttg Leu | ctc Leu 370 | gat Asp | caa Gln | gct Ala | ttg Leu | cgg Arg 375 | caa Gln | cag Gln | aaa Lys | tcg Ser | 1517 |
| tat Tyr 380 | cgc Arg | caa Gln | atg Met | act Thr | ctt Leu 385 | gtt Val | gac Asp | gct Ala | cat His | cct Pro 390 | tgg Trp | cgt Arg | cca Pro | caa Gln | cgc Arg 395 | 1565 |
| Gly | ttg Leu | cct Pro | gaa Glu | cgc Arg 400 | gca Ala | gtc Val | aca Thr | acg Thr | ttg Leu 405 | aga Arg | gct Ala | tgg Trp | ctc Leu | ttt Phe 410 | gaa Glu | 1613 |
| cac His | ttt Phe | ctt Leu | cac His 415 | Pro | tat Tyr | ccg Pro | Ser | gat Asp 420 | gtt Val | gat Asp | aag Lys | cat His | ata Ile 425 | ttg Leu | gcc Ala | 1661 |
| cga Arg | caa Gln | act Thr 430 | ggt Gly | tta Leu | tca Ser | Arg | agt Ser 435 | cag Gln | gta Val | tca Ser | aat Asn | tgg Trp 440 | ttt Phe | att Ile | aat Asn | 1709 |
| gca Ala | aga Arg 445 | gtt Val | agg Arg | cta Leu | tgg Trp | aaa Lys 450 | cca Pro | atg Met | att Ile | gaa Glu | gaa Glu 455 | atg Met | tac Tyr | tgt Cys | gaa Glu | 1757 |
| gaa Glu 460 | aca Thr | aga Arg | agt Ser | gaa Glu | caa Gln 465 | atg Met | gag Glu | att Ile | aca Thr | aac Asn 470 | ccg Pro | atg Met | atg Met | atc Ile | gat Asp 475 | 1805 |
| Thr | Lys | ccg Pro | Asp | Pro 480 | qaA | Gln | Leu | Ile | Arg 485 | Val | Glu | Pro | Ğlu | Ser 490 | Leu | 1853 |
| tcc Ser | tca Ser | ata Ile | gtg Val | aca Thr | aac Asn | cct Pro | aca Thr | tcc Ser | Lys | tcc Ser | Gly | cac His | aac Asn | tca Ser | acc Thr | 1901 |

Page 12

| | MBI16 Sequence | Listing.ST25 |
|-----|----------------|--------------|
| 495 | 500 | 505 |
| | | |

| | 495 | 500 | | 505 | |
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| | gtg aca tac Val Thr Tyr | | | | |
| | ctt ggg tta Leu Gly Leu 545 | | | | |
| | tct cca gtg Ser Pro Val 560 | | | | |
| aga gac cac Arg Asp His | att gaa gaa Ile Glu Glu 575 | gga ccg gtt Gly Pro Val 580 | caa tat tca Gln Tyr Ser | gcg tcg atg Ala Ser Met 585 | tta 2141 Leu |
| | caa gtt cag Gln Val Gln | | | | |
| | cat gat att His Asp Ile | | aaaaga ttag | gaccaa agtta | tcgat 2243 |
| acatattttc | caaaaccgat tc | ggttatgt aad | ggtttag ttag | gataaaa accaa | aattag 2303 |
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2405

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Gln Asn Pro Thr Asp His His His Tyr Asn His Gln Ile Phe Gly Ser 35 40

Asn Ser Asn Met Gly Met Met Ile Asp Phe Ser Lys Gln Gln Gln Ile 50 60

Arg Met Thr Ser Gly Ser Asp His His His His His Gln Thr Ser 65 70 75 80

Gly Gly Thr Asp Gln Asn Gln Leu Leu Glu Asp Ser Ser Ser Ala Met

Arg Leu Cys Asn Val Asn Asn Asp Phe Pro Ser Glu Val Asn Asp Glu 100 105 110

Arg Pro Pro Gln Arg Pro Ser Gln Gly Leu Ser Leu Ser Leu Ser Ser 120

MBI16 Sequence Listing.ST25

Ser Asn Pro Thr Ser Ile Ser Leu Gln Ser Phe Glu Leu Arg Pro Gln 130 135 140

Gln Gln Gln Gly Tyr Ser Gly Asn Lys Ser Thr Gln His Gln Asn 145 150 155 160

Leu Gln His Thr Gln Met Met Met Met Met Met Asn Ser His His Gln 165 170 175

Asn Asn Asn Asn Asn His Gln His His Asn His His Gln Phe Gln 180 185 190

Ile Gly Ser Ser Lys Tyr Leu Ser Pro Ala Gln Glu Leu Leu Ser Glu
195 200 205

Phe Cys Ser Leu Gly Val Lys Glu Ser Asp Glu Glu Val Met Met Met 210 220

Lys His Lys Lys Lys Gln Lys Gly Lys Gln Gln Glu Glu Trp Asp Thr 225 230 235 240

Ser His His Ser Asn Asn Asp Gln His Asp Gln Ser Ala Thr Thr Ser 245 250 255

Ser Lys Lys His Val Pro Pro Leu His Ser Leu Glu Phe Met Glu Leu 260 265 270

Gln Lys Arg Lys Ala Lys Leu Leu Ser Met Leu Glu Glu Leu Lys Arg 275 280 285

Arg Tyr Gly His Tyr Arg Glu Gln Met Arg Val Ala Ala Ala Ala Phe 290 295 300

Glu Ala Ala Val Gly Leu Gly Gly Ala Glu Ile Tyr Thr Ala Leu Ala 305 310 320

Ser Arg Ala Met Ser Arg His Phe Arg Cys Leu Lys Asp Gly Leu Val

Gly Gln Ile Gln Ala Thr Ser Gln Ala Leu Gly Glu Arg Glu Glu Asp 340 345 350

Asn Arg Ala Val Ser Ile Ala Ala Arg Gly Glu Thr Pro Arg Leu Arg 355 360 365

Leu Leu Asp Gln Ala Leu Arg Gln Gln Lys Ser Tyr Arg Gln Met Thr 370 380

Leu Val Asp Ala His Pro Trp Arg Pro Gln Arg Gly Leu Pro Glu Arg 385 390 395 400

Ala Val Thr Thr Leu Arg Ala Trp Leu Phe Glu His Phe Leu His Pro

Tyr Pro Ser Asp Val Asp Lys His Ile Leu Ala Arg Gln Thr Gly Leu
420 425 430

Page 14

| Ser | Arg | Ser 435 | Gln | Val | Ser | Asn | Trp 440 | Phe | Ile | Asn | Ala | Arg 445 | Val | Arg | Leu | |
|------------------------------|------------|---------------------------|------------------|------------|------------|------------|------------|------------------|------------|--------------|------------|------------|------------------|------------|---------------------|-----|
| Trp | Lys 450 | | Met | Ile | Glu | Glu 455 | Met | Tyr | Сув | Glu | Glu 460 | Thr | Arg | Ser | Glu | |
| Gln 465 | Met | Glu | Ile | Thr | Asn 470 | Pro | Met | Met | Ile | Asp 475 | Thr | Lys | Pro | Asp | Pro 480 | |
| qaA | Gln | Leu | Ile | Arg 485 | Val | Glụ | Pro | Glu | Ser 490 | Leu | Ser | Ser | Ile | Val 495 | Thr | |
| Asn | Pro | Thr | Ser 500 | Lys | Ser | Gly | His | Asn 505 | Ser | Thr | His | Gly | Thr 510 | Met | Ser | |
| Leu | Gly | Ser 515 | Thr | Phe | Asp | Phe | Ser 520 | Leu | Tyr | Gly | Asn | Gln 525 | Ala | Val | Thr | |
| Tyr | Ala 530 | Gly | Glu | Gly | Gly | Pro 535 | Arg | Gly | Asp | Val | Ser 540 | Leu | Thr | Leu | Gly | |
| Leu 545 | Gln | Arg | Asn | Asp | Gly 550 | Asn | Gly | Gly | Val | Ser 555 | Leu | Ala | Leu | Ser | Pro 560 | |
| Val | Thr | Ala · | Gln | Gly 565 | Gly | Gln | Leu | Phe | Tyr 570 | Gly | Arg | Asp | His | Ile 575 | Glu | |
| Glu | Gly | Pro | Val 580 | Gln | Tyr | Ser | Ala | Ser 585 | Met | Leu | Авр | Авр | Авр 590 | Gln | Val | |
| Gln | Asn | Leu 595 | Pro | Tyr | Arg | Asn | Leu 600 | Met | Gly | Ala | Gln | Leu 605 | Leu | His | Asp | |
| Ile | Val 610 | | | | | | | | | | | | | | | |
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| | | | gtg Val | | | | | | | | | | | | | 103 |
| ttg Leu | gga Gly | tta Leu | 999 Gly 25 | ctc Leu | agc Ser | ctc Leu | ggt Gly | ggt Gly 30 | ggc Gly | gcg Ala | tgg Trp | aaa Lys | gag Glu 35 | cgt Arg | G 1 Å 333 | 151 |
| agg | att | ctt | act | gct | aag | gat | ttt | cct | | gtt ige 1 | | tct | aaa | cgc | tct | 199 |

| Ara | Ile | Leu | Thr | Ala | Lvs | Asp | | | | | List Glv | | | | Ser | | |
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| 3 | | 40 | | | -,- | _F | 45 | | | | , | 50 | -1- | 5 | | | |
| | | | tcc Ser | | | | | | | | | | | | | 24 | 7 |
| | 55 | - | | - | | 60 | | | - | | 65 | **** | 502 | - | 02 | | |
| | | | tgg Trp | | | | | | | | | | | | | 29 | 5 |
| 70 | *** | 027 | | | 75 | | | 200 | | 80 | | 7.011 | 502 | 204 | 85 | | |
| | | | gct Ala | | | | | | | | | | | | | 34 | 3 |
| | | 01 | niu | 90 | 270 | 7.1.0 | | , Lug | 95 | 014 | 014 | 017 | n.op | 100 | 014 | | |
| | | | gtg Val | | | | | | | | | | | | | 39 | 1 |
| -,- | 2,0 | , | 105 | 2,0 | | or, | 014 | 110 | -,- | p | | 002 | 115 | 2,0 | | | |
| | | | gtt Val | | | | | | | | | | | | | 43 | 9 |
| | 110 | 120 | ,,, | U111 | O.J | 204 | 125 | | • | 2,5 | | 130 | | | or ₁ | | |
| gtt Val | ggt | ata | ggc Gly | aga Arg | aaa Lvs | gtg | gat | atg Met | aga Arg | gct | cat | tcg | tct | tac | gaa | 48 | 7 |
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| | | | gaa Glu | | | | | | | | | | | | | 58 | 3 |
| | -75 | | 010 | 170 | • | -75 | | | 175 | 200 | | | 017 | 180 | 502 | | |
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<213> Arabidopsis thaliana .

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Trp Lys Glu Arg Gly Arg Ile Leu Thr Ala Lys Asp Phe Pro Ser Val $_{35}$ 40 45

MBI16 Sequence Listing.ST25 Gly Ser Lys Arg Ser Ala Glu Ser Ser Ser His Gln Gly Ala Ser Pro Pro Arg Ser Ser Gln Val Val Gly Trp Pro Pro Ile Gly Leu His Arg Met Asn Ser Leu Val Asn Asn Gln Ala Met Lys Ala Ala Arg Ala Glu Glu Gly Asp Gly Glu Lys Lys Val Val Lys Asn Gly Glu Leu Lys Asp Val Ser Met Lys Val Asn Pro Lys Val Gln Gly Leu Gly Phe Val Lys Val Asn Met Asp Gly Val Gly Ile Gly Arg Lys Val Asp Met Arg Ala 130 135 140 His Ser Ser Tyr Glu Asn Leu Ala Gln Thr Leu Glu Glu Met Phe Phe Gly Met Thr Gly Thr Thr Cys Arg Glu Thr Val Lys Pro Leu Arg Leu Leu Asp Gly Ser Ser Asp Phe Val Leu Thr Tyr Glu Asp Lys Gly Ile Gly Cys Leu Leu Glu Met Phe His Gly Glu Cys Leu Ser Thr Arg 200 <210> <211> 1065 <212> DNA <213> Arabidopsis thaliana <220> CDS <221> <222> (188)..(760) <223> G482 <400> 15 togacccacg cgtccggaca cttaacaatt cacaccttct ctttttactc ttcctaaaac 60 120 cctaaatttc ctcgcttcag tcttcccact caagtcaacc accaattgaa ttcgatttcg aatcattgat ggaaatgatt tgaaaaaaga gtaaagttta tttttttatt ccttgtaatt 180 ttcagaa atg ggg gat tcc gac agg gat tcc ggt gga ggg caa aac ggg Met Gly Asp Ser Asp Arg Asp Ser Gly Gly Gln Asn Gly 229 aac aac cag aac gga cag tcc tcc ttg tct cca aga gag caa gac agg Asn Asn Gln Asn Gly Gln Ser Ser Leu Ser Pro Arg Glu Gln Asp Arg 277 ttc ttg ccg atc gct aac gtc agc cgg atc atg aag aag gcc ttg ccc Phe Leu Pro Ile Ala Asn Val Ser Arg Ile Met Lys Lys Ala Leu Pro 325 gcc aac gcc aag atc tct aaa gat gcc aaa gag acg atg cag gag tgt Ala Asn Ala Lys Ile Ser Lys Asp Ala Lys Glu Thr Met Gln Glu Cys 50 55 60373 gtc tcc gag ttc atc agc ttc gtc acc gga gaa gca tct gat aag tgt 421 Page 17

| MBI16 Sequence Listing.ST25 | |
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| cag aag gag aag agg aag acg atc aac gga gac gat ttg ctc tgg gct | 469 |
| Gln Lys Glu Lys Arg Lys Thr Ile Asn Gly Asp Asp Leu Leu Trp Ala | |
| 80 85 90 | |
| atg act act cta ggt ttt gag gat tat gtt gag cca ttg aaa gtt tac Met Thr Thr Leu Gly Phe Glu Asp Tyr Val Glu Pro Leu Lys Val Tyr | 517 |
| 95 100 105 110 | |
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| Leu Gln Arg Phe Arg Glu Ile Glu Gly Giu Arg Thr Gly Leu Gly Arg | |
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| Asp Gly Gly Gly Phe Tyr Gly Gly Gly Gly Met Gln Tyr His Gin | |
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| His His Gln Phe Leu His Gln Gln Asn His Met Tyr Gly Ala Thr Gly 160 165 170 | |
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Page 19

| Pro | Lys | Gly | Glu 120 | Lys | Thr | Asn | MBI Trp | 16 S Ile 125 | eque Met | nce His | List Glu | ing. Tyr | ST25 Arg 130 | Leu | Ala | |
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| gat Asp | tgg Trp 150 | gtt Val | ctc Leu | tgc Cys | cgg Arg | att Ile 155 | tac Tyr | aac Asn | aaa Lys | aaa Lys | gga Gly 160 | gct Ala | acc Thr | gag Glu | agg Arg | 715 |
| cgg Arg 165 | gga Gly | cca Pro | ccg Pro | cct Pro | ccg Pro 170 | gtt Val | gtt Val | tac Tyr | ggc Gly | gac Asp 175 | gaa Glu | atc Ile | atg Met | gag Glu | gag Glu 180 | 763 |
| aag Lys | ccg Pro | aag Lys | gtg Val | acg Thr 185 | gag Glu | atg Met | gtt Val | atg Met | cct Pro 190 | ccg Pro | ccg Pro | ccg Pro | caa Gln | cag Gln 195 | aca Thr | 811 |
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| act Thr | acg Thr | gat Asp 215 | tcg Ser | agt Ser | tgc Cys | tcg Ser | gag Glu 220 | cag Gln | gtg Val | gtg Val | tcg Ser | ccg Pro 225 | gag Glu | ttc Phe | acg Thr | 907 |
| agc Ser | gag Glu 230 | gtt Val | cag Gln | agc Ser | gag Glu | ccc Pro 235 | aag Lys | tgg Trp | aaa Lys | gat Asp | tgg Trp 240 | tcg Ser | gcc Ala | gta Val | agt Ser | 955 |
| aat Asn 245 | gac Asp | aat Asn | aac Asn | aat Asn | acc Thr 250 | ctt Leu | gat Asp | ttt Phe | G1y 999 | ttt Phe 255 | aat Asn | tac Tyr | att Ile | gat Asp | gcc Ala 260 | 1003 |
| acc Thr | gtg Val | gat Asp | aac Asn | gcg Ala 265 | ttt Phe | gga Gly | gga Gly | gga Gly | 999 Gly 270 | agt Ser | agt Ser | aàt Asn | cag Gln | atg Met 275 | ttt Phe | 1051 |
| ccg Pro | cta Leu | cag Gln | gat Asp 280 | atg Met | ttc Phe | atg Met | tac Tyr | atg Met 285 | cag Gln | aag Lys | cct Pro | tac Tyr | tag | | | 1093 |
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| tggc | aaca | icg a | gaco | gttt | t at | atgg | tcaa | tga | gtgt | gcc | gatt | cggc | ca t | taga | tttct | 1213 |
| gtto | agto | tt c | gttt | atto | t at | agac | cgtc | cga | tttc | aga | tcat | ccct | aa t | cgga | cggtg | 1273 |
| gtcg | ttgg | at g | tato | agta | g tg | tatt | actg | tgt | tagg | tag | aaga | aaat | cc a | cttg | ttctt | 1333 |
| aaat | tggc | at a | aaag | tcag | a ag | ctaa | tatt | tat | atgt | gcc | gcaa | tcaa | tt t | aata | ttttc | 1393 |
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<212> PRT <213> Arabidopsis thaliana

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Ser Ile Ala Val Pro Ile Ile Ala Glu Ile Asp Leu Tyr Lys Tyr Asp 35 40 45

MBI16 Sequence Listing.ST25
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Arg Ser Ala Gly Ser Gly Tyr Trp Lys Ala Thr Gly Ala Asp Lys Pro

Ile Gly Leu Pro Lys Pro Val Gly Ile Lys Lys Ala Leu Val Phe Tyr 100 110

Ala Gly Lys Ala Pro Lys Gly Glu Lys Thr Asn Trp Ile Met His Glu 115 120 125

Tyr Arg Leu Ala Asp Val Asp Arg Ser Val Arg Lys Lys Asn Ser 130 135 140

Leu Arg Leu Asp Asp Trp Val Leu Cys Arg Ile Tyr Asn Lys Lys Gly 145 150 150

Ala Thr Glu Arg Arg Gly Pro Pro Pro Pro Val Val Tyr Gly Asp Glu 165 170 175

Ile Met Glu Glu Lys Pro Lys Val Thr Glu Met Val Met Pro Pro Pro 180 185 190

Pro Gln Gln Thr Ser Glu Phe Ala Tyr Phe Asp Thr Ser Asp Ser Val

Pro Lys Leu His Thr Thr Asp Ser Ser Cys Ser Glu Gln Val Val Ser 210 215 220

Pro Glu Phe Thr Ser Glu Val Gln Ser Glu Pro Lys Trp Lys Asp Trp 225 230 235 240

Ser Ala Val Ser Asn Asp Asn Asn Asn Thr Leu Asp Phe Gly Phe Asn 245 250 255

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Page 22

ggt tct gct tcg ggc tct acg tac aac aac aac aac gag atg atc aag Gly Ser Ala Ser Gly Ser Thr Tyr Asn Asn Asn Asn Glu Met Ile Lys 260 270

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| | | | | | | | | | | | | | | agc Ser | | 1140 |
| tcg Ser | tat Tyr | gaa Glu | gat Asp | cta Leu 325 | tgt Cys | gac Asp | ttg Leu | agg Arg | 999 330 | gac Asp | ttg Leu | tgg Trp | gac Asp | ttc Phe 335 | taa | 1188 |
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| Glu | Ile | Ile 35 | Thr | Cys | Tyr | Leu | Lys 40 | Glu | Lys | Val | Leu | Asn 45 | Ser | Arg | Phe~ | |
| Thr | Ala 50 | Val | Ala | Met | Gly | Glu 55 | Ala | Asp | Leu | Asn | Lys 60 | Сув | Glu | Pro | Trp | |
| Asp 65 | Leu | Pro | Lys | Arg | Ala 70 | Lys | Met | Gly | Glu | Lys 75 | Glu | Phe | Туг | Phe | Phe 80 . | |
| Cys | Gln | Arg | Asp | Arg 85 | Lys | туг | Pro | Thr | Gly 90 | Met | Arg | Ťhr | Asn | Arġ 95 | Ala | |
| Thr | Glu | Ser | Gly 100 | Tyr | Trp | Lуз | Ala | Thr 105 | Gly | Lys | Asp | Lys | Glu 110 | Ile | Phe | |
| Lys | Gly | Lys 115 | Gly | Суз | Leu | Val | Gly 120 | Met | Lys | Lys | Thr | Leu 125 | Val | Phe | Tyr | |
| Arg | Gly 130 | Arg | Ala | Pro | Lys · | Gly 135 | Glu | Lys | Thr | Asn | Trp 140 | Val | Met | His | Glu | |
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Page 23

MBI16 Sequence Listing.ST25 165 170 Thr Thr Gln Pro Met Thr Arg Ile Pro Val Glu Asp Phe Thr Arg 180 185 190 Met Asp Ser Leu Glu Asn Ile Asp His Leu Leu Asp Phe Ser Ser Leu Pro Pro Leu Ile Asp Pro Ser Phe Met Ser Gln Thr Glu Gln Pro Asn Phe Lys Pro Ile Asn Pro Pro Thr Tyr Asp Ile Ser Ser Pro Ile Gln Pro His His Phe Asn Ser Tyr Gln Ser Ile Phe Asn His Gln Val Phe Gly Ser Ala Ser Gly Ser Thr Tyr Asn Asn Asn Asn Glu Met Ile Lys Met Glu Gln Ser Leu Val Ser Val Ser Gln Glu Thr Cys Leu Ser Ser Asp Val Asn Ala Asn Met Thr Thr Thr Thr Glu Val Ser Ser Gly Pro Val Met Lys Gln Glu Met Gly Met Gly Met Val Asn Gly Ser Lys Ser Tyr Glu Asp Leu Cys Asp Leu Arg Gly Asp Leu Trp Asp Phe 325 330 335 <210> 21 <211> 890 <212> DNA <213> Arabidopsis thaliana <220> <221> CDS <222> (55)..(738) <223> G545 gcaaccttca aactaaaact cgagagacaa gaaatcctca gaatctttaa ctta atg 57 Met gcg ctc gag gct ctt aca tca cca aga tta gct tct ccg att cct cct Ala Leu Glu Ala Leu Thr Ser Pro Arg Leu Ala Ser Pro Ile Pro Pro 105 ttg ttc gaa gat tct tca gtc ttc cat gga gtc gag cac tgg aca aag Leu Phe Glu Asp Ser Ser Val Phe His Gly Val Glu His Trp Thr Lys 153 ggt aag cga tct aag aga tca aga tcc gat ttc cac cac caa aac ctc Gly Lys Arg Ser Lys Arg Ser Arg Ser Asp Phe His His Gln Asn Leu 201

60

act gag gaa gag tat cta gct ttt tgc ctc atg ctt ctc gct cgc gac Thr Glu Glu Glu Tyr Leu Ala Phe Cys Leu Met Leu Leu Ala Arg Asp

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| | | | | gac Asp | | | | | | | | | | | | 345 | |
| cac His | aag Lys | gca Ala 100 | agc Ser | cac His | cgt Arg | aag Lys | aac Asn 105 | tta Leu | tca Ser | cag Gln | act Thr | ctc Leu 110 | tcc Ser | ggc Gly | gga Gly | 393 | |
| | | | | tca Ser | | | | | | | | | | | | 441 | |
| | | | | aaa Lys | | | | | | | | | | | | 489 | |
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| | | | | aac Asn | | | | | | | | | | | | . 585 | |
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| tgag | jagtt | gt g | gtagg | gaatt | t gt | tgac | :tgta | cat | acca | aat | tgga | cttt | ga d | tgat | tccaa | 838 | |
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| Pro | Leu | Phe | Glu 20 | Asp | Ser | Ser | Val | Phe 25 | His | Gly | Val | Glu | | Trp | Thr | | |
| Lys | Gly | Lys 35 | Arg | Ser | Lys | Arg | Ser 40 | Arg | Ser | qaA | Phe | His 45 | His | Gln | Asn | | |
| Leu | Thr 50 | Glu | Glu | Glu | Tyr | Leu 55 | Ala | Phe | Cys | Leu | Met 60 | Leu | Leu | Ala | Arg | | |
| Asp 65 | Asn | Arg | Gln | Pro | Pro 70 | Pro | Pro | Pro | Ala | Val 75 | Glu | Lys | Leu | Ser | Tyr 80 | | |

PCT/US00/31458 WO 01/36598

MBI16 Sequence Listing.ST25 Lys Cys Ser Val Cys Asp Lys Thr Phe Ser Ser Tyr Gln Ala Leu Gly Gly His Lys Ala Ser His Arg Lys Asn Leu Ser Gln Thr Leu Ser Gly Gly Gly Asp Asp His Ser Thr Ser Ser Ala Thr Thr Thr Ser Ala Val Thr Thr Gly Ser Gly Lys Ser His Val Cys Thr Ile Cys Asn Lys Ser Phe Pro Ser Gly Gln Ala Leu Gly Gly His Lys Arg Cys His Tyr Glu Gly Asn Asn Asn Ile Asn Thr Ser Ser Val Ser Asn Ser Glu Gly Ala Gly Ser Thr Ser His Val Ser Ser Ser His Arg Gly Phe Asp Leu Asn Ile Pro Pro Ile Pro Glu Phe Ser Met Val Asn Gly Asp Asp Glu Val Met Ser Pro Met Pro Ala Lys Lys Pro Arg Phe Asp Phe Pro Val Lys Leu Gln Leu 225 <210> 23 <211> 1413 <212> DNA <213> Arabidopsis thaliana <220> <221> <222> (86)..(1168) G561 <400> 23 aatttgtttt tttttctttt gtgggttcaa ttcgaattgt tttccctgag actcaagtta 60 ctgtgtcatt actctgcatt gagca atg ggt agc aac gaa gaa gga aac ccc Met Gly Ser Asn Glu Glu Gly Asn Pro 160 act aac aac tct gat aag cca tcg caa gct gct gct cct gag cag agt Thr Asn Asn Ser Asp Lys Pro Ser Gln Ala Ala Ala Pro Glu Gln Ser 10 15 20 25 aat gtt cat gtg tat cat cat gac tgg gct gct atg cag gca tat tat Asn Val His Val Tyr His His Asp Trp Ala Ala Met Gln Ala Tyr Tyr 30 35 40208 ggg cct aga gtt ggt ata cct caa tat tac aac tca aat ttg gcg cct Gly Pro Arg Val Gly Ile Pro Gln Tyr Tyr Asn Ser Asn Leu Ala Pro 45 50 55 256 ggt cat gct cca ccg cct tat atg tgg gcg tct cca tcg cca atg atg Gly His Ala Pro Pro Pro Tyr Met Trp Ala Ser Pro Ser Pro Met Met 60 65 70

304

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| 90 95 100 105 | |
| tot caa toa goa tot gga gtt aca acc cot ttg acc att gat goa coa | 448 |
| Ser Gln Ser Ala Ser Gly Val Thr Thr Pro Leu Thr Ile Asp Ala Pro | |
| 110 115 120 | |
| gct aat tca gct gga aac tca gat cat ggg ttc atg aaa aag ctg aaa | 496 |
| Ala Asn Ser Ala Gly Asn Ser Asp His Gly Phe Met Lys Lys Leu Lys 125 130 135 | |
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| 140 145 150 | |
| got gaa cat ago ago agt gaa cat agg agt tot cag ago too gag aat | 592 |
| Ala Glu His Ser Ser Ser Glu His Arg Ser Ser Gln Ser Ser Glu Asn | |
| 155 160 165 | |
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| Asp Gly Ser Ser Asn Gly Ser Asp Gly Asn Thr Thr Gly Gly Glu Gln 170 175 180 185 | |
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| 190 195 200 | |
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| 235 240 245 | |
| gtt aaa cga gag aag aga aaa cag tca aac cga gaa tct gct agg agg | 880 |
| Val Lys Arg Glu Lys Arg Lys Gln Ser Asn Arg Glu Ser Ala Arg Arg | |
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| tca aga ctg agg aag cag gct gaa aca gaa caa cta tct gtc aaa gtt | 928 |
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| 285 290 295 | |
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| Leu Asn Asn Glu Ser Glu Lys Leu Arg Leu Glu Asn Glu Ala Ile Leu | |
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| 315 320 325 | |
| cga gtt gat aag aac aac tot gta toa ggt agc aaa act gtg cag cat | 1120 |
| Arg Val Asp Lys Asn Asn Ser Val Ser Gly Ser Lys Thr Val Gln His | 1110 |
| 330 335 340 345 | |
| caa ctg tta aat gca agt ccg ata acc gat cct gtc gcg gct agc tga | 1168 |
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| aggagacttt ttgtttttat tcttagattt gtagctctct gcatagtgag cataaattga | 1288 |
| Page 27 | |

MBI16 Sequence Listing.ST25

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Gln Tyr Tyr Asn Ser Asn Leu Ala Pro Gly His Ala Pro Pro Pro Tyr 50 60

Met Trp Ala Ser Pro Ser Pro Met Met Ala Pro Tyr Gly Ala Pro Tyr 65 70 80

Pro Pro Phe. Cys Pro Pro Gly Gly Val Tyr Ala His Pro Gly Val Gln 85 90 95

Met Gly Ser Gln Pro Gln Gly Pro Val Ser Gln Ser Ala Ser Gly Val 100 105 110

Thr Thr Pro Leu Thr Ile Asp Ala Pro Ala Asn Ser Ala Gly Asn Ser 115 120 125

Asp His Gly Phe Met Lys Lys Leu Lys Glu Phe Asp Gly Leu Ala Met 130 135 140

Ser Ile Ser Asn Asn Lys Val Gly Ser Ala Glu His Ser Ser Ser Glu 145 150 150 160

His Arg Ser Ser Gln Ser Ser Glu Asn Asp Gly Ser Ser Asn Gly Ser 165 170 175

Asp Gly Asn Thr Thr Gly Gly Glu Gln Ser Arg Arg Lys Arg Arg Gln
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Gln Arg Ser Pro Ser Thr Gly Glu Arg Pro Ser Ser Gln Asn Ser Leu 195 200 205

Pro Leu Arg Gly Glu Asn Glu Lys Pro Asp Val Thr Met Gly Thr Pro 210 215 220

Val Met Pro Thr Ala Met Ser Phe Gln Asn Ser Ala Gly Met Asn Gly 225 230 240

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| Asp | Asn | Glu | Ile | Lys 105 | Asn | Tyr | | 16 S Asn | | | | | | Lys 115 | Leu | |
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| | gct Ala | | | | | | | | | | | | | | | 547 |
| | acc Thr 150 | | | | | | | | | | | | | | | 595 |
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| | ttc Phe | | | | | | | | | | | | | | | 691 |
| | ttg Leu | | | | | | | | | | | | | | | 739 |
| | caa Gln | | | | | | | | | | | | | | | 787 |
| | 999 Gly 230 | | | | | | | | | | | | | | | 835 |
| | gta Val | | | | | | | | | | | | | | | 883 |
| gat Asp | ttt Phe | tta Leu | 999 Gly | ttg Leu 265 | gca Ala | aag Lys | aaa Lys | gag Glu | acc Thr 270 | act Thr | tct Ser | ctt Leu | ttg Leu | ggc Gly 275 | ttt Phe | 931 |
| | agc Ser | | | | | taa | tatt | gtca | aa t | ttta | aggcg | jt aa | actgl | acaa | ì | 982 |
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| Trp | Thr | Lys | Glu 20 | Glu | Asp | Glu | Arg | Leu 25 | Val | Ala | Tyr | Ile | Lys 30 | Ala | His | |

Gly Glu Gly Cys Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Leu Arg 35 40 45

Cys Gly Lys Ser Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp 50 55

| | | | | | | | MRT | 16 S | emie | nce ' | List | ing. | ST25 | | | | |
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| Leu | His | Ser | Leu | Leu 85 | Gly | Asn | Lys | Trp | Ser 90 | Leu | Ile | Ala | Gly | Arg 95 | Leu | | |
| Pro | Gly | Arg | Thr 100 | Asp | Åsn | Glu | Ile | Lys 105 | Asn | Tyr | Trp | Asn | Thr 110 | His | Ile | | |
| Arg | Arg | Lys 115 | Leu | Ile | Asn | Arg | Gly 120 | Ile | Asp | Pro | Thr | Ser 125 | His | Arg | Pro | | |
| Ile | Gln 130 | Glu | Ser | Ser | Ala | Ser 135 | Gln | Ąsp | Ser | Lys | Pro 140 | Thr | Gln | Leu | Glu | | |
| Pro 145 | Val | Thr | Ser | Asn | Thr 150 | Ile | Asn | Ile | Ser | Phe 155 | Thr | Ser | Ala | Pro | Lys 160 | | |
| Val | Glu | Thr | Phe | His 165 | Glu | Ser | Ile | Ser | Phe 170 | Pro | Gly | Lys | Ser | Glu 175 | Lys | | |
| Ile | Ser | Met | Leu 180 | Thr | Phe | Lys | Glu | Glu 185 | Lys | Asp | Glu | Сув | Pro 190 | Val | Gln | | |
| 3lu | | Phe 195 | Pro | Asp | Leu | Asn | Leu 200 | Glu | Leu | Arg | Ile | Ser 205 | Leu | Pro | Asp | | |
| Asp | Val 210 | Asp | Arg | Leu | Gln | Gly 215 | His | Gly | Lys | Ser | Thr 220 | Thr | Pro | Arg | Cys | | |
| Phe 225 | Lys | Сув | Ser | Leu | Gly 230 | Met | .Ile | Asn | Gly | Met 235 | Glu | Суз | Arg | Cys | Gly 240 | | |
| Arg | Met | Arg | Суз | Asp 245 | Val | Val | Gly | Gly | Ser 250 | Ser | Lys | Gly | Ser | Asp [.] 255 | Met | | |
| Ser | Asn | Gly | Phe 260 | Asp | Phe | Leu | Gly | Leu 265 | Ala | Lys | Ļув | Glu | Thr 270 | Thr | Ser | | |
| Leu | | Gly 275 | | Arg | Ser | | Glu 280 | Met | Lys | | | | | | | | |
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| <212 | ?> I | ANC | | _ | | | | | | | | | | | | | |
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| - 66- | (| | | | | , | | | | | | | | | | | |
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| | • | | | | | | MBI | 16 S | eque | nce : | List | ing. | ST25 | | | |
|------------------------------|------------------|---------------------------|------------|------------|------------------|------------------|------------------|------------------|------------|------------------|------------------|------------------|------------|------------|------------------|-----|
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| ggt Gly | gac Asp 50 | agg Arg | tgg Trp | gaa Glu | ctg Leu | ata Ile 55 | gct Ala | G1y 999 | agg Arg | atc Ile | cca Pro 60 | gga Gly | aga Arg | acc Thr | gct Ala | 192 |
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| Thr | Ser | Ser | Ser 20 | Glu | Glu | Val _. | Ser | Ser 25 | Leu | Glu | Trp | Glu | Val 30 | Val | Asn | |
| Met | Ser | Gln 35 | Glu | Glu | Glu | Asp | Leu 40 | Val | Ser | Arg | Met | Нів 45 | Lув | Leu | Val | |
| Gly | Asp 50 | Arg | Trp | Glu | Leu | Ile 55 | Ala | Gly | Arg | Ile | Pro 60 | Gly | Arg | Thr | Ala | |
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| gct Ala | ctt Leu | tgt Cys 35 | cct Pro | tac Tyr | att Ile | ggt Gly | cta Leu 40 | cct Pro | agt Ser | ttt Phe | cta Leu | gac Asp 45 | cac His | aac Asn | gag Glu | 144 |
| acc Thr | tct Ser 50 | gga Gly | ccc Pro | gat Asp | ccg Pro | acc Thr 55 | cga Arg | cac His | gct Ala | ctċ Leu | tct Ser 60 | acg Thr | tca Ser | gcg Ala | agt Ser | 192 |
| ctt Leu 65 | gct Ala | aac Asn | gag Glu | ttg Leu | atc Ile 70 | ccg Pro | gtg Val | gtt Val | cgg Arg | ttc Phe 75 | tcg Ser | gat Asp | ctt Leu | ccg Pro | acc Thr 80 | 240 |
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| tgt tta gac cgt Cys Leu Asp Arg 115 | tgg atc gtt Trp Ile Val | gac tac aa Asp Tyr As 120 | ac aag atg aaa an Lys Met Lys 125 | tgt ccg gtt 384 Cys Pro Val |
| tgt cgg cac cgg Cys Arg His Arg 130 | | | | |
| ggt tct ggt tca Gly Ser Gly Ser 145 | | | | |
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| Ile Leu Lys Ile 20 | Leu Tyr Val | Ile Gly Ph | ne Phe Arg Asp | Met Val Asp 30 |
| Ala Leu Cys Pro 35 | Tyr Ile Gly | Leu Pro Se | er Phe Leu Asp . 45 | His Asn Glu |
| Thr Ser Gly Pro | Asp Pro Thr 55 | Arg His Al | la Leu Ser Thr 60 | Ser Ala Ser |
| Leu Ala Asn Glu 65 | Leu Ile Pro 70 | Val Val Ar | rg Phe Ser Asp 75 | Leu Pro Thr 80 |
| Asp Pro Glu Asp | Cys Cys Thr 85 | Val Cys Le | | Glu Ser Asp 95 |
| Asp Lys Val Arg | Gln Leu Pro | Lys Cys Gl 105 | ly His Val Phe | His His His 110 |
| Cys Leu Asp Arg 115 | Trp Ile Val | Asp Tyr As | sn Lys Met Lys 125 | Cys Pro Val |
| Cys Arg His Arg | Phe Leu Pro 135 | Lys Glu Ly | s Tyr Thr Gln | Cys Asp Trp |
| Gly Ser Gly Ser 145 | Asp Trp Phe 150 | Ser Asp Gl | lu Val Glu Ser 155 | Thr Asn |
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MBI16 Sequence Listing.ST25

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| ttc | tgaa | ac t | gttg | gagtt | c tt | gtga | aagg | g aaa | ataaa | aaa | | | g at | | | | 176 |
| gaa Glu | gat Asp | cta Leu | ggt Gly | ttg Leu 10 | agc Ser | cta Leu | agc Ser | tta Leu | 999 Gly 15 | ttt Phe | tca Ser | caa Gln | aat Asn | cac His 20 | aat Asn | . | 224 |
| cct Pro | ctt Leu | cag Gln | atg Met 25 | aat Asn | ctg Leu | aat Asn | cct Pro | aac Asn 30 | tct Ser | tca Ser | tta Leu | tca Ser | aac Asn 35 | aat Asn | Leu | | 272 |
| cag Gln | aga Arg | ctc Leu 40 | cca Pro | tgg Trp | aac Asn | caa Gln | aca Thr 45 | ttc Phe | gat Asp | cct Pro | aca Thr | tca Ser 50 | gat Asp | ctt Leu | cgc Arg | : | 320 |
| aag Lys | ata Ile 55 | gac Asp | gtg Val | aac Asn | agt Ser | ttt Phe 60 | cca Pro | tca Ser | acg Thr | gtt Val | aac Asn 65 | tgc Cys | gag Glu | gaa Glu | gac | : | 368 |
| | | | tcg Ser | | | | | | | | | | | | | | 416 |
| | | | gag Glu | | | | | | | | | | | | | | 464 |
| | | | gac Asp 105 | | | | | | | | | | | | | | 512 |
| tca Ser | gat Asp | gaa Glu 120 | gaa Glu | gaa Glu | gac Asp | 999 Gly | ggc Gly 125 | gaa Glu | acg Thr | tcg Ser | agg Arg | aag Lys 130 | aag Lys | ctc Leu | agg Arg | ! | 560 |
| tta Leu | tca Ser 135 | aaa Lys | gat Asp | cag Gln | tct Ser | gct Ala 140 | ttt Phe | ctc Leu | gaa Glu | gag Glu | act Thr 145 | ttc Phe | aaa Lys | gaa Glu | Cac | | 608 |
| aac Asn 150 | act Thr | ctc Leu | aat Asn | ecc Pro | aaa Lys 155 | cag Gln | aag Lys | cta Leu | gct Ala | ttg Leu 160 | gct Ala | aag Lys | aag Lys | ctg Leu | aac Asn 165 | l | 656 |
| ttg Leu | acg Thr | gca Ala | aga Arg | caa Gln 170 | gtg Val | gaa Glu | gtg Val | tgg Trp | ttc Phe 175 | caa Gln | aac Asn | aga Arg | aga Arg | gct Ala 180 | aga | Ī | 704 |
| acc | aag Lys | tta Leu | aag Lys 185 | caa Gln | acg Thr | gag Glu | gta Val | gat Asp 190 | tgc Cys | gaa Glu | tac Tyr | ttg Leu | aaa Lys 195 | cgg Arg | tgc Cys | : | 752 |
| | | | cta Leu | | | | | | | | | | | | | | 800 |
| gag Glu | ctt Leu 215 | cga Arg | act Thr | ctc Leu | aag Lys | ctg Leu 220 | tct Ser | cca Pro | caa Gln | ttc Phe | tac Tyr 225 | ggt Gly | cag Gln | atg Met | act Thr | ; | 848 |
| cca Pro 230 | cca Pro | act Thr | aca Thr | ctc Leu | atc Ile 235 | atg Met | tgt Cys | cct Pro | tcg Ser | tgc Cys 240 | gag Glu | cgt Arg | gta Val | gct Ala | ggt Gly 245 | , | 896 |
| | | | tcg Ser | | | | | | | | | | | | | | 944 |

| | | | | | | | MBI | 16 S | eque | nce | List | ina | ST25 | | |
|------------------------------|--------------|---------------------------|-------------------|------------|------------|-------------|------------|------------|------------|--------------|------------|-------------------|------------|------------|------------|
| ccg Pro | tgg Trp | att Ile | gct Ala 265 | tgt Cys | gct Ala | ggt Gly | caq | ata | act | cat | aaa | cta | aat | ttt Phe | gaa Glu |
| gcc Ala | ttg Leu | cgt Arg 280 | cca Pro | cga Arg | tcg Ser | taa | ttt | ttag | tgg 1 | tgggg | ggaa | 99 9 ¹ | tgtt | ttgg | 3 |
| ttt | ttc | att a | atcgi | tata | at a | gtcta | atct | g tg | tggg | gtca | ttg | taati | ttt | ggat | gattgg |
| cctt | ctc | atg a | aacta | agtca | at a | tgtal | tgate | g ca | acct | taaa | aat | attt | caa 🤉 | gtago | caaaac |
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| Ser | Gln | Asn | His 20 | Asn | Pro | Leu | Gln | Met 25 | Asn | Leu | Asn | Pro | Asn 30 | Ser | Ser |
| Leu | Ser | Asn 35 | Asn | Leu | Gln | Arg | Leu 40 | Pro | Trp | Asn | Gln | Thr 45 | Phe | Asp | Pro |
| Thr | Ser 50 | Asp | Leu | Arg | Lys | Ile 55 | Ąsp | Val | Asn | Ser | Phe 60 | Pro | Ser | Thr | Val |
| Asn 65 | Cys | Glu | Glu | qėA | Thr 70 | Gly | Val | Ser | Ser | Pro 75 | Asn | Ser | Thr | Ile | Ser 80 |
| Ser | Thr | Ile | Ser | Gly 85 | Lys | Arg | Ser | Glu | Arg 90 | Glu | Gly | Ile | Ser | Gly 95. | Thr |
| Gly | Val | Gly | Ser 100 | Gly | Ąsp | Asp | His | Asp 105 | Glu | Ile | Thr | Pro | Asp 110 | Arg | Gly |
| Tyr | Ser | Arg 115 | Gly | Thr | Ser | Asp | Glu 120 | Glu | Glu | Asp | Gly | Gly 125 | Glu | Thr | Ser |
| Arg | Lув 130 | Lys | Leu | Arg | Leu | Ser .135 | Lys | Asp | Gln | Ser | Ala 140 | Phe | Leu | Glu | Glu |
| Thr 145 | | Lys | Glu | His | Asn 150 | Thr | Leu | Asn | Pro | Lys 155 | Gln | Lys | Leu | Ala | Leu 160 |
| Ala | Lys | Lys | Leu | Asn 165 | Leu | Thr | Ala | Arg | Gln 170 | Val | Glu | Val | Trp | Phe 175 | Gln |
| Asn | Arg | Arg | Ala 180 | Arg | Thr | Lys | Leu | Lys 185 | Gln | Thr | Glu | Val | Asp 190 | Суз | Glu |
| Tyr | Leu | Lys 195 | Arg | Сув | Val | Glu | Lys 200 | Leu | Thr | Glu | Glu | Asn 205 | Arg | Arg | Leu |
| Gln | Lys | Glu | Ala | Met | Glu | Leu | Arg | Thr | | Lys age : | _ | Ser | Pro | Gln | Phe |

MBI16 Sequence Listing.ST25 210 215 220

Tyr Gly Gln Met Thr Pro Pro Thr Thr Leu Ile Met Cys Pro Ser Cys 225 230 235 240 Glu Arg Val Ala Gly Pro Ser Ser Ser Asn His His His Asn His Arg Pro Val Ser Ile Asn Pro Trp Ile Ala Cys Ala Gly Gln Val Ala His Gly Leu Asn Phe Glu Ala Leu Arg Pro Arg Ser <210> 33 <211> 1249 <212> DNA Arabidopsis thaliana <213> <220> <221> CDS <222> (82)..(918) <223> G394 <400> 33 gaaattotta acaaacaatt ttottoataa tattaattot caagatotta aagattatat taatacgaag agaaaattca a atg ggt ctt gat gat tca tgc aac aca ggt Met Gly Leu Asp Asp Ser Cys Asn Thr Gly ctt gtt ctt ggt tta ggc ctc tca cca acg cct aat aat tac aat cat Leu Val Leu Gly Leu Gly Leu Ser Pro Thr Pro Asn Asn Tyr Asn His 159 gcc atc aag aaa tct tcc tcc act gtg gac cat cgt ttc atc agg ctc Ala Ile Lys Lys Ser Ser Ser Thr Val Asp His Arg Phe Ile Arg Leu 30 35 40207 gat ccg tcg ttg act cta agc cta tcc ggt gag agc tac aag atc aag Asp Pro Ser Leu Thr Leu Ser Leu Ser Gly Glu Ser Tyr Lys Ile Lys 45 50 55 255 act ggt gcc ggc gcc ggc gac caa att tgc cgg cag acc tcg tcc cac Thr Gly Ala Gly Ala Gly Asp Gln Ile Cys Arg Gln Thr Ser Ser His 60 65 70303 agc ggc atc tca tct ttc tcg agc gga agg gta aag aga gaa aga gaa Ser Gly Ile Ser Ser Phe Ser Ser Gly Arg Val Lys Arg Glu Arg Glu 351 atc tcc ggc ggc gat gga gaa gaa gag gcg gag gag acg acg gag aga Ile Ser Gly Gly Asp Gly Glu Glu Glu Ala Glu Glu Thr Thr Glu Arg 399 gtg gtg tgt tcg aga gtg agt gat gat cat gac gat gaa gaa ggt gtt Val Val Cys Ser Arg Val Ser Asp Asp His Asp Asp Glu Glu Gly Val 447 agt gct cgt aaa aag ctt aga ctc act aaa caa caa tct gct ctt ctc Ser Ala Arg Lys Lys Leu Arg Leu Thr Lys Gln Gln Ser Ala Leu Leu 495 543 gaa gat aac ttc aaa ctt cat agc acc ctt aat ccc aag caa aaa caa Glu Asp Asn Phe Lys Leu His Ser Thr Leu Asn Pro Lys Gln Lys Gln 145 150 get ett geg aga cag etg aat eta agg eet aga caa gtt gaa gtg tgg 591

Ala Leu Ala Arg Gln Leu Asn Leu Arg Pro Arg Gln Val Glu Val Trp

Page 36

| 155 | 160 | MBI16 Sequence | | |
|--|---|--|---|----------------|
| | 160 | 165 | | |
| | | | caa aca gaa gtg gat Gln Thr Glu Val Asp 185 | 639 |
| | | | acg gat gag aat aga Thr Asp Glu Asn Arg 200 | 687 |
| | | | tta aaa ttg tct caa Leu Lys Leu Ser Gln 215 | 735 |
| | | | act atg tgc cct tct Thr Met Cys Pro Ser 230 | 783 |
| | | | gat acg acg gcg gtt Asp Thr Thr Ala Val 250 | 831 |
| | | | gtc aca aag cct cgt Val Thr Lys Pro Arg 265 | 879 |
| | | cct tct gca gca Pro Ser Ala Ala 275 | tgt tag ttacttatta Cys | 928 |
| gttatttaat tctt | ttgtt ggtttt | tttt ttgtttctta | aatcaaatta ggaattagtt | 988 |
| agaagataaa tccca | ngggaa aaaata | ttac gttgaaattg | gggggaaatg gggtatagto | 1048 |
| tttatagata agagi | | | | 1108 |
| cccacagaca agact | eccea acgaci | ccac tttattttc | ggtgggattg ttggttgatg | 3 1100 |
| | | | agagaaaaaa tgacgaatat | |
| aagaaaaaaa aata | gtttgt aattac | aggt ttaaatatgt | | 1168 |
| aagaaaaaaa aata | stttgt aattac | aggt ttaaatatgt | agagaaaaaa tgacgaatat | 1168 |
| aagaaaaaaa aatag gtattatctt gtttt aaataataaa tatat <210> 34 <211> 278 <212> PRT | stttgt aattac | aggt ttaaatatgt | agagaaaaaa tgacgaatat | 1168 a 1228 |
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Glu Glu Glu Ala Glu Glu Thr Thr Glu Arg Val Val Cys Ser Arg Val

| | | | 100 | | | | MBI | 16 Se 105 | equei | nce l | List | ing. | T25 110 | | | |
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| Arg | Leu 130 | Thr | Lys | Gln | Gln | Ser 135 | Ala | Leu | Leu | Glu | Asp 140 | Asn | Phe | Lys | Leu | |
| His 145 | Ser | Thr | Leu | Asn | Pro 150 | Lys | Gln | Lys | Gln | Ala 155 | Leu | Ala | Arg | Gln | Leu 160 | |
| Asn | Leu | Arg | Pro | Arg 165 | Gln | Val | Glu | Val | Trp 170 | Phe | Gln | Asn | Arg | Arg 175 | Ala | |
| Arg | Thr | Lys | Leu 180 | Lys | Gln | Thr | Glu | Val 185 | Asp | Сув | Glu | Phe | Leu 190 | Lys | Lys | |
| Cys | Сув | Glu 195 | Thr | Leu | Thr | Asp | Glu 200 | Asn | Arg | Arg | Leu | Gln 205 | Lys | Glu | Leu | |
| Gln | Asp 210 | | Lys | Ala | Leu | Lys 215 | Leu | Ser | Gln | Pro | Phe 220 | Tyr | Met | His | Met | |
| Pro 225 | Ala | Ala | Thr | Leu | Thr 230 | Met | Cys | Pro | Ser | Cys 235 | Glu | Arg | Leu | Gly | Gly 240 | |
| Gly | Gly | Val | Gly | Gly 245 | Asp | Thr | Thr | Ala | Val 250 | Asp | Glu | Glu | Thr | Ala 255 | Lys | |
| Gly | Ala | Phe | Ser 260 | Ile | Val | Thr | Lys | Pro 265 | Arg | Phe | Tyr | Asn | Pro 270 | Phe | Thr | |
| Asn | Pro | Ser 275 | Ala | Ala | Сув | | | | | | | | | | | |
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101

149

197

tac caa acc aac ccg atg age acc act gct gct act gta gca gga ggt Tyr Gln Thr Asn Pro Met Ser Thr Thr Ala Ala Thr Val Ala Gly Gly 10 15 20

gcg gca caa cca ggc cag ctg gcg ttc cac cag atc cat cag cag cag Ala Ala Gln Pro Gly Gln Leu Ala Phe His Gln Ile His Gln Gln Gln 25

cag cag caa cag ctg gca cag cag ctt caa gca ttt tgg gag aac caa Gln Gln Gln Gln Leu Ala Gln Gln Leu Gln Ala Phe Trp Glu Asn Gln 40 45 50 55

| WO 01/36598 | PCT/US00/31458 |
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| atc tcg gct gag gcg ccg gtc gtg ttt gca agg gcc tgt gag atg ttc Ile Ser Ala Glu Ala Pro Val Val Phe Ala Arg Ala Cys Glu Met Phe 90 95 100 | 341 |
| atc ctg gag ctg aca ctc agg tcg tgg aac cac act gag gag aat aag Ile Leu Glu Leu Thr Leu Arg Ser Trp Asn His Thr Glu Glu Asn Lys 105 110 115 | 389 |
| agg cgg acg ttg cag aag aac gat att gct gct gct gtg act aga acc Arg Arg Thr Leu Gln Lys Asn Asp Ile Ala Ala Ala Val Thr Arg Thr 120 125 130 135 | 437 |
| gat att ttt gat ttc ctt gtg gat att gtt ccc cgg gag gat ctc cga Asp Ile Phe Asp Phe Leu Val Asp Ile Val Pro Arg Glu Asp Leu Arg 140 145 150 | 485 |
| gat gaa gtc ttg gga agt att ccg agg ggc act gtc ccg gaa gct gct Asp Glu Val Leu Gly Ser Ile Pro Arg Gly Thr Val Pro Glu Ala Ala 155 160 165 | 533 |
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| atttactgtg ttttttattc ggttttcgct atcgaactgt gaaatggaaa tggatggaga | 965 |
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| cttatttgtg gggatgaatt tgaaattata agagatatgc aaacattttg tttgagtaaa | 1085 |
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| Phe 65 | Lys | Asn | His | Ser | Leu 70 | Pro | Leu | Ala | Arg | Ile 75 | Lys | Lys | Ile | Met | Lys 80 | | |
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| Ala | Arg | Ala | Сув 100 | Glu | Met | Phe | Ile | Leu 105 | Glu | Leu | Thr | Leu | Arg 110 | Ser | Trp | | |
| Asn | His | Thr 115 | Glu | Glu | Asn | Lys | Arg 120 | Arg | Thr | Leu | Gln | Lys 125 | Asn | Asp | Ile | | |
| Ala | Ala 130 | | Val | Thr | Arg | Thr 135 | Asp | Ile | Phe | Asp | Phe 140 | Leu | Val | Asp | Ile | | |
| Val 145 | | Arg | Glu | Asp | Leu 150 | Arg | Asp | Glu | Val | Leu 155 | Gly | Ser | Ile | Pro | Arg 160 | | |
| Gly | Thr | Val | Pro | Glu 165 | | Ala | Ala | Ala | Gly 170 | Tyr | Pro | туг | Gly | Tyr 175 | Leu | | |
| Pro | Ala | Gly | Thr 180 | | Pro | Ile | Gly | 7 Asr 185 | Pro | Gly | Met | : Val | . Met 190 | Gly | Asn | | |
| Pro | Gly | Gly 195 | | туг | Pro | Pro | 200 | n Pro | туг | Met | : Gly | / Glr 205 | n Pro | Met | Trp | | |
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| | | | | | | | | | | | | | | | tttct | | 180 |
| ta | gtgg | gttt | ttg | ttgt | tgt (| tgtt | gtgg | tc t | ctct | g at Me 1 | g at t Il | t ac e Th | t ga r Gl | a ct u Le 5 | t gag u Glu | | 234 |
| at Me | g 99 t Gl | g aa y Ly | a gg s Gl 10 | y Gl | g ag u Se | t gag | g ct u Le | t ga u Gl 15 | и ге | t gg u Gl | t ct y Le | a gg u Gl | g ct y Le 20 | u se | t ctt r Leu | | 282 |

| WO 01/36598 | PCT/US00/31458 |
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| atg gat gga gtt gct ata gga aga aaa gtg gat ttg aat gct cat tct Met Asp Gly Val Ala Ile Gly Arg Lys Val Asp Leu Asn Ala His Ser 135 140 145 150 | 666 |
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Lys Asp Phe Pro Ser Val Gly Ser Lys Arg Ala Ala Asp Ser Ala Ser 50 55 60

His Ala Gly Ser Ser Pro Pro Arg Ser Ser Gln Val Val Gly Trp Pro 65 70 75 80

Pro Ile Gly Ser His Arg Met Asn Ser Leu Val Asn Asn Gln Ala Thr 85 90 95

Lys Ser Ala Arg Glu Glu Glu Glu Ala Gly Lys Lys Lys Val Lys Asp 100 105 110

Asp Glu Pro Lys Asp Val Thr Lys Lys Val Asn Gly Lys Val Gln Val 115 120 125

Gly Phe Ile Lys Val Asn Met Asp Gly Val Ala Ile Gly Arg Lys Val 130 135 140

Asp Leu Asn Ala His Ser Ser Tyr Glu Asn Leu Ala Gln Thr Leu Glu 145 150 150 160

Asp Met Phe Phe Arg Thr Asn Pro Gly Thr Val Gly Leu Thr Ser Gln 165 170 175

Phe Thr Lys Pro Leu Arg Leu Leu Asp Gly Ser Ser Glu Phe Val Leu 180 185

Thr Tyr Glu Asp Lys Glu Gly Asp Trp Met Leu Val Gly Asp Val Pro 195 200 205

Trp Arg Met Phe Ile Asn Ser Val Lys Arg Leu Arg Val Met Lys Thr 210 215 220

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| tgt cgt aaa tgc gcg tcg cag ccg atc cct gct ccg att atc acc gaa Cys Arg Lys Cys Ala Ser Gln Pro Ile Pro Ala Pro Ile Ile Thr Glu 30 35 40 | 207 |
| ctc gat ttg tac cga tat gat cct tgg gac ctt ccc gac atg gct ttg Leu Asp Leu Tyr Arg Tyr Asp Pro Trp Asp Leu Pro Asp Met Ala Leu 45 50 55 60 | 255 |
| tac ggt gaa aag gag tgg tat ttt ttc tca cca aga gat cga aag tat Tyr Gly Glu Lys Glu Trp Tyr Phe Phe Ser Pro Arg Asp Arg Lys Tyr 65 70 75 | 303 |
| cca aac ggt tca aga ccc aac cgt gca gct ggt act gga tat tgg aaa Pro Asn Gly Ser Arg Pro Asn Arg Ala Ala Gly Thr Gly Tyr Trp Lys 80 85 90 | 351 |
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| ttc tga gttgtcacgt gcgattagag ttagtggaaa gtggaaacta tcactgtctg Phe | 887 |
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PCT/US00/31458 WO 01/36598

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Arg Tyr Asp Pro Trp Asp Leu Pro Asp Met Ala Leu Tyr Gly Glu Lys 50 55 60

Glu Trp Tyr Phe Phe Ser Pro Arg Asp Arg Lys Tyr Pro Asn Gly Ser 65 70 75 80

Arg Pro Asn Arg Ala Ala Gly Thr Gly Tyr Trp Lys Ala Thr Gly Ala 85 90 95

Asp Lys Pro Ile Gly Arg Pro Lys Pro Val Gly Ile Lys Lys Ala Leu 100 105 110

Val Phe Tyr Ser Gly Lys Pro Pro Asn Gly Glu Lys Thr Asn Trp Ile 115 120 125

Met His Glu Tyr Arg Leu Ala Asp Val Asp Arg Ser Val Arg Lys Lys 130 135 140

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Lys Gly Val Ile Glu Lys Arg Arg Ser Asp Ile Glu Asp Gly Leu Lys 165 170 175

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Gly Ser Glu Gln Ala Val Ser Pro Glu Phe Thr Cys Ser Asn Gly Arg

Leu Ser Asn Ala Leu Asp Phe Pro Phe Asn Tyr Val Asp Ala Ile Ala

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| | • | • | | | | | | | | | | | | |
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| 1 | GIU GI | 5 | Oly | V 4. | • | • | 10 | | - 1 | <i>,</i> | | 15 | | |
| Val Val | Asp Le | | Pro | Gly | Phe | Arg 25 | Phe | His | Pro | Thr | Asp 30 | Glu | Glu | |
| Ile Ile | Thr Hi 35 | is Tyr | Leu | Lys | Glu 40 | Lys | Val | Phe | Asn | Ile 45 | Arg | Phe | Thr | |
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| Gln Arg | Asp A | rg Lys 85 | Tyr | Pro | Thr | Gly | Met 90 | Arg | Thr | Asn | Arg | Ala 95 | Thr | |
| Val Ser | | yr Trp 00 | Lys | Ala | Thr | Gly 105 | Lys | Asp | Lys | Glu | Ile 110 | Phe | Arg | |
| Gly Lys | Gly C: 115 | ys Leu | Val | Gly | Met 120 | Lys | Lys | Thr | Leu | Val 125 | Phe | Tyr | Thr | |
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| Arg Leu 145 | Asp G | ly Lys | Tyr 150 | Ser | туг | His | Asn | Leu 155 | Pro | Lys | Thr | Ala | Arg 160 | |
| Asp Glu | Trp V | al Val 165 | Сув | Arg | Val | Phe | His 170 | Lys | Asn | Ala | Pro | Ser 175 | Thr | |

| Thr I | ie ' | | Thr 180 | Thr | Lув | Gln | | l6 Se Ser 185 | | | | | | Asp | Asn | |
|------------------------------------|-------------------|-----------------------|-------------------|-------------------|-------------------|-------------------|-------------------|---------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------------|
| Ile A | | His 195 | Leu | Leu | Asp | Phe | Ser 200 | Ser | Leu | Pro | Pro | Leu 205 | Ile | Asp | Pro · | |
| Gly P | he 210 | Leu | Gly | Gln | Pro | Ala 215 | Gln | Ala | Ser | Pro | Val 220 | Pro | Val | Asn | Asn | |
| Thr I 225 | le | Ser | Asn | Leu | Ser 230 | Pro | Pro | Ser | Tyr | Asn 235 | Arg | Thr | Ser | Arg | Gln 240 | |
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| aag a Lys 1 | agg Arg | aag Lys 115 | cta Leu | tta Leu | aga Arg | aaa Lys | 999 Gly 120 | att Ile | gat Asp | ccg Pro | gcg Ala | act Thr 125 | cat His | cga Arg | cct Pro | 384 |
| atc a Ile A | aac Asn 130 | gag Glu | acc Thr | aaa Lys | act Thr | tct Ser 135 | caa Gln | gat Asp | tcg Ser | tct Ser | gat Asp 140 | tct Ser | agt Ser | aaa Lys | aca Thr | 432 |
| gag g Glu i 145 | gac Asp | cct Pro | ctt Leu | gtc Val | aag Lys 150 | att Ile | ctc Leu | tct Ser | ttt Phe | ggt Gly 155 | cct Pro | cag Gln | ctg Leu | gag Glu | aaa Lys 160 | 480 |
| ata (| gca Ala | aat Asn | ttc Phe | 999 Gly 165 | gac Asp | gag Glu | aga Arg | att Ile | caa Gln 170 | Lys | aga Arg | gtt Val | gag Glu | tac Tyr 175 | Ser | 528 |

Page 47

MBI16 Sequence Listing.ST25

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| cca Pro | cca Pro | tgg Trp 195 | caa Gln | gac Asp | aag Lys | ctc Leu | cat His 200 | gat Asp | gag Glu | agg Arg | aac Asn | cta Leu 205 | agg Arg | ttt Phe | G1 y 999 | 624 |
| aga Arg | gtg Val 210 | aag Lys | tat Tyr | agg Arg | tgc Cys | agt Ser 215 | gcg Ala | Cya Cya | cgt Arg | ttt Phe | gga Gly 220 | ttc Phe | 61 y 999 | aac Asn | ggc Gly | 672 |
| aag Lys 225 | gag Glu | tgt Cys | agc Ser | tgt Cys | aat Asn 230 | aat Asn | gtg Val | aaa Lys | tgt Cys | caa Gln 235 | aca Thr | gag Glu | gac Asp | agt Ser | agt Ser 240 | 720 |
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| ttc Phe | ttg Leu | ggt Gly | cta Leu 260 | Asn | aac Asn | act Thr | agg Arg | gtt Val 265 | ttg Leu | gat Asp | ttt Phe | agc Ser | act Thr 270 | hen | gaa Glu | 816 |
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Leu Lys Arg Gly Asn Phe Thr Leu Glu Glu Asp Asp Leu Ile Ile Lys 65 70 75 80

Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu Ile Ala Thr Arg Leu 85 90 95

Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Val

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Glu Asp Pro Leu Val Lys Ile Leu Ser Phe Gly Pro Gln Leu Glu Lys 145 150 155 160

Page 48

MBI16 Sequence Listing.ST25

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aag aac tac tgg aac aca cat ata aag agg aag ctt ttg agc aaa ggg

Page 49

| | | | | | | | MBI: | 16 Se | eque | nce 1 | List | ing. | ST25 | | | |
|--------------------------|-------------------|--------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-----------------------|-------------------|-------------------|-------------------|-----|
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| ttg Leu | aag Lys | aaa Lys | aca Thr 140 | aag Lys | gac Asp | caa Gln | att Ile | gta Val 145 | aaa Lys | gat Asp | gtt Val | tct Ser | ttt Phe 150 | gtg Val | aca Thr | 485 |
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| aaa Lys 185 | ata Ile | ggc Gly | cca Pro | gat Asp | ttg Leu 190 | aat Asn | ctt Leu | gag Glu | ctt Leu | agg Arg 195 | atc Ile | agt Ser | cca Pro | cca Pro | tgg Trp 200 | 629 |
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| gtt Val | ggt Gly 250 | Tyr | gac Asp | ttc Phe | ttg Leu | ggt Gly 255 | Leu | aag Lys | aca Thr | aga Arg | att Ile 260 | Leu | gat Asp | ttt Phe | cga Arg | 821 |
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| Gly | / Glu | 3 Gly 35 | у Сув | Trp | Arg | Ser | Let 40 | Pro | Arg | Ala | a Ala | Gl ₃ 45 | / Lev | Lev | Arg | |
| Cys | 5 Gly 50 | Lys | s Sei | Сув | a Arg | J Let 55 | ı Arç | Trp | ıle | e Ası | Туі 60 | . Lev | a Arg | Pro | Asp | |
| Let 65 | ı Lya | a Ar | g Gly | / Ası | n Phe 70 | e Thi | c His | aA s | Gl: | 1 Asj 75 | o Glu | ı Leı | 1 Ile | e Ile | e Lys 80 | |
| Le | . Hi: | s Se | r Lei | ı Lev | ı Gly | y Ası | n Ly | s Tr | 9 Se | r Lei | ı Ile | e Ala | a Ala | 95 | g Leu | |

MBI16 Sequence Listing.ST25

| | | | | | | | | | -4-0. | | | 3 • • | | | | |
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| Val : | Lys | Asp | Val | Ser | Phe 150 | Val | Thr | Lys | Phe | Glu 155 | Glu | Thr | Asp | Lув | Ser 160 | |
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| Сув | Thr 210 | Ala | Ser | Arg | Phe | Tyr 215 | Met | Glu | Asn | Asp | Met 220 | Glu | Сув | Ser | Ser | |
| Glu 225 | Thr | Val | Lys | Сув | Gln 230 | Thr | Glu | Asn | Ser | Ser 235 | Ser | Ile | Ser | Tyr | Ser 240 | |
| Ser | Ile | Asp | Ile | Ser 245 | | Ser | Asn | Val | Gly 250 | Tyr | Asp | Phe | Leu | Gly 255 | Leu | |
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| tac Tyr | atc Ile | ggt | cta Leu | cca Pro | agt Ser | ttt Phe | ctt | gac Asp | cac His | aac Asn | gag Glu | acc Thr | tct Ser | cga Arg 50 | tcc Ser | 200 |
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| sp | Pro | Thr | Arg 55 | Leu | Ala | Leu | MBI: Ser | 16 Se Thr 60 | equei Ser | nce l Ala | List: Thr | ing.: Leu | ST25 Ala 65 | Asn | Glu | |
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| gc Cys | tgc Cys 85 | acg Thr | gtt Val | tgc Cys | tta Leu | tcc Ser 90 | gat Asp | ttt Phe | gta Val | tcc Ser | gac Asp 95 | gat Asp | aag Lys | att Ile | aga Arg | 344 |
| cag 31n 100 | ctg Leu | ccg Pro | aag Lys | tgt Cys | gga Gly 105 | cac His | gtg Val | ttt Phe | cat His | cat His 110 | Arg | tgt Cys | tta Leu | gac Asp | cgt Arg 115 | 392 |
| tgg Trp | atc Ile | gtt Val | gac Asp | tgt Cys 120 | aat Asn | aag Lys | ata Ile | acg Thr | tgc Cys 125 | ccg Pro | att Ile | tgt Cys | cgg Arg | aac Asn 130 | cgg Arg | 440 |
| ttc Phe | tta Leu | ccg Pro | gag Glu 135 | gaa Glu | aag Lys | tcc Ser | acg Thr | ccg Pro 140 | Pne | gat Asp | tgg Trp | ggt Gly | act Thr 145 | Ser | gat Asp | 488 |
| tgg Trp | ttt Phe | aga Arg 150 | Asp | gaa Glu | gtg Val | gag Glu | agt Ser 155 | Thr | aac Asn | taa | taa | tgat | ggt | ttta | ctttta | 541 |
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| Pro | Gl: | u As | р Су | в Су: 85 | s Thi | r Val | . Cyı | s Lei | u Se: 90 | r As | p Ph | e Va | l Se | r Ası 95 | p Asp | |
| Ly | s Il | e Ar | g Gl: 10 | n Le 0 | u Pro | o Lys | : Су: | s Gl | y Hi 5 | в Va | l Ph | e Hi | s Hi 11 | s Arg | g Cys | |
| Le | u As | p Ar 11 | | p Il | e Va | l Asp | Cy 12 | s As 0 | n Ly | s Il | e Th | r Cy 12 | s Pr 5 | o Il | e Cys | |
| Ar | g As | n Ar | g Ph | e Le | u Pr | o Glu | ı Gl | u Ly | s Se | r Th | r Pr 14 | o Ph | е Ав | p Tr | p Gly | |

MBI16 Sequence Listing.ST25
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Page 53

798

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MBI16 Sequence Listing.ST25

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| agt Ser | cca Pro 235 | ccg Pro | acc Thr | aca Thr | Leu l | ttg a Leu 1 240 | atg Met | tgt Cys | cca Pro | ser (| tgt Cys 245 | gaa (Glu / | egt g Arg V | gtg g Val A | icc lla | 894 |
| gga Gly 250 | cca Pro | tcc Ser | tca Ser | Ser | aac (Asn 1 255 | cac His | aac Asn | cag Gln | Arg | tct Ser 260 | gtc Val | tca (Ser) | ttg a Leu S | ser i | cca Pro 265 | 942 |
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| tct Ser | taa | ctt | taatg | gct 9 | cttc | tatg | g gt | tgtg | ıtgtg | ggt | catt | gta | cttt | ttagi | at | 1046 |
| tatt | gac | tct · | cagct | aatg | t at | cctt | aaaa | gco | tttt | tct | actt | ttaa | at t | tact | ttaat | 1106 |
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| 1 | | | | 5 | | | | | 10 | | | Ser | | | | |
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| Leu 65 | Pro | Th: | c Thr | . Val | Asp 70 | Leu | Glu | Glu | Glu | Thr 75 | Gly | Val | Ser | Ser | Pro 80 | |
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| Gl | y Gl 13 | | u Th | r Cys | a Arg | Lys 139 | Lys ; | Let | ı Arg | J Lev | 140 | r Lys | Авр | Gln | Ser | |
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| | | | | | | | | | eque | | | | | | | |
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| Glu | Val | Asp 195 | Cys | Glu | Tyr | Leu | Lys 200 | Arg | Сув | Val | Glu | Lys 205 | Leu | Thr | Glu | |
| | Asn 210 | Arg | Arg | Leu | Glu | Lys 215 | Glu | Ala | Ala | Glu | Leu 220 | Arg | Ala | Leu | Lys | |
| Leu 225 | Ser | Pro | Arg | Leu | Tyr 230 | Gly | Gln | Met | Ser | Pro 235 | Pro | Thr | Thr | Leu | Leu 240 | |
| Met | Cys \ | Pro | Ser | Сув 245 | Glu | Arg | Val | Ala | Gly 250 | Pro | Ser | Ser | Ser | Asn 255 | His | |
| Asn | Gln | Arg | Ser 260 | Val | Ser | Leu | Ser | Pro 265 | Trp | Leu | Gln | Met | Ala 270 | His | Gly | |
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| _ | | | | | | | | | | | | | | | gaaga | 119 |
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| gaa Glu | gcg Ala | gtg Val | cct Pro 20 | agt Ser | atg Met | tca Ser | gta Val | tcg Ser 25 | ccg Pro | ccg Pro | gat Asp | agt Ser | gta Val 30 | acg Thr | tcg Ser | 215 |
| tcg Ser | ttt Phe | caa Gln 35 | ttg Leu | gac Asp | ttt Phe | 999 999 | att Ile 40 | aaa Lys | agt Ser | tat Tyr | ggt Gly | tat Tyr 45 | gag Glu | aga Arg | aga Arg | 263 |
| agc Ser | aat Asn 50 | aag Lys | aga Arg | gat Asp | att | gat Asp 55 | gat Asp | gaa Glu | gtg Val | gag Glu | aga Arg 60 | tca Ser | gcc Ala | tca Ser | aga Arg | 311 |
| gcc Ala 65 | agc Ser | aac Asn | gaa Glu | gac Asp | aac Asn 70 | gat Asp | gac Asp | gag Glu | aat Asn | gga Gly 75 | tcc Ser | act Thr | agg Arg | aag Lys | aaa Lys 80 | 359 |
| ctt Leu | aga Arg | ctc Leu | tcc Ser | aaa Lys 85 | gac Asp | caa Gln | tct Ser | gct Ala | ttt Phe 90 | ctt Leu | gaa Glu | gac | ago Ser | tto Phe 95 | aaa Lys | 407 |
| gaa Glu | cac His | agt Ser | acc Thr | Leu | aat Asn | cct Pro | aaa Lys | cag Gln 105 | Lys | att | gca Ala | ttg Leu | gcg Ala 110 | nys | cag Gln | 455 |

Page 55

MBI16 Sequence Listing.ST25

| ttg aa | | | | | | | MBI | 16 Se | quer | ice I | ist | ing.S | T25 | | | |
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| Leu As | sn L | et Leu 115 | cgt Arg | cct Pro | cgt Arg | cag Gln | gtt Val 120 | gaa Glu | gtc Val | tgg Trp | ttt Phe | caa Gln 125 | aac Asn | aga Arg | cga Arg | 503 |
| gcc ag Ala Ai 13 | gg a rg T 30 | aca Thr | aag Lys | ctg Leu | aag Lys | caa Gln 135 | acg Thr | gaa Glu | gtg Val | gac Asp | tgt Cys 140 | gaa Glu | tac Tyr | cta Leu | aag Lys | 551 |
| aga to Arg C | gc t ys C | tgt Cys | gag Glu | tca Ser | cta Leu 150 | acc Thr | gaa Glu | gaa Glu | aac Asn | cgg Arg 155 | agg Arg | ctt Leu | caa Gln | aaa Lys | gag Glu 160 | 599 |
| gtt aa Val Ly | aa g ys C | gaa Glu | ttg Leu | aga Arg 165 | acc Thr | ttg Leu | aag Lys | act Thr | tcc Ser 170 | aca Thr | ccc Pro | ttt Phe | tac Tyr | atg Met 175 | caa Gln | 647 |
| ctt co Leu P | cg g | gcc Ala | act Thr 180 | act Thr | ctc Leu | act Thr | atg Met | tgc Cys 185 | cct Pro | tct Ser | tgt Cys | gaa Glu | cgt Arg 190 | gtt Val | gcc Ala | 695 |
| act to Thr S | Ser 1 | gca Ala 195 | gca Ala | cag Gln | ccc Pro | tcc Ser | acg Thr 200 | tca Ser | gct Ala | gcc Ala | cac His | aac Asn 205 | ctc Leu | tgt Cys | ttg Leu | 743 |
| tcc a Ser T | hr i | tca Ser | tca Ser | ttg Leu | att Ile | ccg Pro 215 | gtt Val | aag Lys | cct Pro | cgg | ccg Pro 220 | gcc Ala | aaa Lys | caa Gln | gtt Val | 791 |
| tca t Ser 225 | ga i | aago | cacc | tgc | gaaal | taca | gt t | tgag | caaa | c gg | gcgg | ccgc | tct | agac | agg | 847 |
| cctcq | gtac | cg g | gatc | ctct | ag c | taga | gctt | t cg | ttcg | tatc | atc | ggtt | tċg | acaa | cgttcg | 907 |
| tcaag | | | | | | | | | | | | | | | • | 937 |
| | | | | | | | | | | | | | | | | |
| <210><211><211><212><213> | > 2 > P | 2 25 RT Lrab | idop | sis | thal | iana | | | | | | | | | | |
| <211><212> | > 2 > P > A | 25 RT | idop | sis | thal | iana | | | | | | | | | | |
| <211><212><213> | > 2 > P > A > 5 | 25 PRT Tab | Ī | | | | | . Val | Glu 10 | Glu | Glu | : Glu | Glu | Glu 15 | Glu | |
| <211><212><212><213><400> | > 2 > P > A > 5 | 225 PRT Tab 52 Leu | Gly | Ala 5 | Ala | Thr | Val | | 10 | | | | | 13 | | |
| <211><212><213> 400 Met F | > 2 > P > A > 5 Pro | 225 PRT Tab 52 Leu Val | Gly Pro | Ala 5 Ser | Ala | Thr | Val | . Ser 25 | Pro | Pro |) Asr | Ser | Va] 30 | . Thr | Ser | |
| <211><212><213><213><400> Met I Glu I Ser I Ser I | > 2 > P > A > 5 Pro | 225 PRT Trab 52 Leu Val Gln 35 | Gly Pro 20 | Ala 5 Ser | Ala Met | Thr Ser | Val | . Ser 25 | Pro | Pro Tyr | Asp | Ser Tyr 45 | Val 30 | . Thr | Ser | |
| <211><212><213><400> Met I Glu J Ser I | > 2 > P > A > 5 Pro Ala Phe Asn 50 | 225 PRT Arab 52 Leu Val Gln 35 | Pro 20 Leu | Ala 5 Ser Asr | Ala Met | Thr Ser Gly Asr 55 | Val | Ser 25 Lys | Pro | Pro | Gly | Tyr 45 Ser | Val 30 Glu | Thr Arg | Ser | |
| <211><212><213><400><203><400>Met I1Glu JSer IAla 365 | > 2 > P > A > 5 Pro Ala Phe Asn 50 | PRT PRT PRT PRT PRT PRT PRT PRT PRT PRT | Gly Pro 20 Leu Arc | Ala 5 Ser Asp Asp | Ala Met Phe Asr 70 | Thr Ser Gly Asp 55 | Val | Ser 25 Lys Glu | Pro Ser Val | Glu Gly 75 | Gly Arc 60 Sen | y Tyr 45 Ser | Val 30 Glu Ala | Thr Arg | Ser Arg Arg | |
| <pre><211> <212> <213> <400> Met E 1 Glu J Ser I Ala : 65</pre> | > 2 > P > A > 5 Pro Ala Phe Asn 50 Ser | PRT PRT PRT PRT PRT PRT PRT PRT PRT PRT | Pro 20 Leu Arg | Alas Ser Asp Asp Asp Asp Asp | Ala Met Phe Asr 70 | Thr Ser Gly S5 1 Asp | Val | Ser 25 25 25 25 Clubs Glubs Gl | Pro Pro Val | Pro Pro Glu | Gly Sen | y Tyr 45 Ser Thr | Val 30 Glu Ala Argo Sea | The Arc | Ser Arg Arg Lys | |

MBI16 Sequence Listing.ST25

Ala Arg Thr Lys Leu Lys Gln Thr Glu Val Asp Cys Glu Tyr Leu Lys Arg Cys Cys Glu Ser Leu Thr Glu Glu Asn Arg Arg Leu Gln Lys Glu Val Lys Glu Leu Arg Thr Leu Lys Thr Ser Thr Pro Phe Tyr Met Gln Leu Pro Ala Thr Thr Leu Thr Met Cys Pro Ser Cys Glu Arg Val Ala Thr Ser Ala Ala Gln Pro Ser Thr Ser Ala Ala His Asn Leu Cys Leu Ser Thr Ser Ser Leu Ile Pro Val Lys Pro Arg Pro Ala Lys Gln Val Ser 225 <210> <211> 927 <212> DNA <213> Arabidopsis thaliana <221> (37) .. (861) <222> G393 <223> <400> 53 aaattttaaa ggcatatttt tttcatttcg actcag atg ggt ttt gat gat aca Met Gly Phe Asp Asp Thr tgc aac aca ggt ctt gtt ctt gga tta ggt ccc tca cca att tca aat Cys Asn Thr Gly Leu Val Leu Gly Leu Gly Pro Ser Pro Ile Ser Asn 102 aat tac aat agt acc atc aga caa tcc tcc gtt tac aag ctc gag ccg Asn Tyr Asn Ser Thr Ile Arg Gln Ser Ser Val Tyr Lys Leu Glu Pro 25 30 35 150 tcg ttg act cta tgc ctc tcg ggc gat ccc tcg gtt acc gtg gtg acc Ser Leu Thr Leu Cys Leu Ser Gly Asp Pro Ser Val Thr Val Val Thr 40 45 50198 gga gct gac cag cta tgc cgt cag acg tca tct cac agc gga gtc tct Gly Ala Asp Gln Leu Cys Arg Gln Thr Ser Ser His Ser Gly Val Ser 246 tct ttc tca agc ggg agg gtg gtg aaa aga gag aga gac ggt ggc gaa Ser Phe Ser Ser Gly Arg Val Val Lys Arg Glu Arg Asp Gly Glu Glu Glu 65 294 gag tcg ccg gag gag gaa gag atg acg gag aga gtt ata agt gat tac Glu Ser Pro Glu Glu Glu Glu Met Thr Glu Arg Val Ile Ser Asp Tyr 342 cat gaa gat gaa gat ggt att agt gct aga aaa actt agg ctt acg His Glu Asp Glu Glu Gly Ile Ser Ala Arg Lys Lys Leu Arg Leu Thr 390 aaa caa caa tot got ott ott gag gaa ago tto aag gat cat ago acc

Page 57

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|-------------------|--------------------------|--------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|---------------------|-------------------|-------------------|-------------------|-------------------|-----|
| Lys | Gln 120 | Gln | Ser | Ala | Leu | Leu 125 | MBI1 Glu | l6 Se Glu | equer Ser | ice I Phe | Listi Lys 130 | ing.S Asp | T25 His | Ser | Thr | |
| ctt Leu 135 | aat Asn | ccc Pro | aaa Lys | caa Gln | aag Lys 140 | caa Gln | gtt Val | ctg Leu | gct Ala | aga Arg 145 | cag Gln | ctg Leu | aat Asn | cta Leu | agg Arg 150 | 486 |
| cct Pro | aga Arg | caa Gln | gtt Val | gaa Glu 155 | gta Val | tgg Trp | ttt Phe | caa Gln | aat Asn 160 | aga Arg | aga Arg | gcc Ala | agg Arg | aca Thr 165 | aag Lys | 534 |
| ctg Leu | aag Lys | caa Gln | aca Thr 170 | gaa Glu | gta Val | gat Asp | tgt Cys | gag Glu 175 | ttt Phe | ttg Leu | aag Lys | aag Lys | tgt Cys 180 | tgt Cys | gaa Glu | 582 |
| aca Thr | tta Leu | gca Ala 185 | gat Asp | gag Glu | aac Asn | ata Ile | aga Arg 190 | ctt Leu | cag Gln | aaa Lys | gag Glu | att Ile 195 | caa Gln | gaa Glu | ctc Leu | 630 |
| aaa Lys | acc Thr 200 | Leu | aaa Lys | ttg Leu | act Thr | cag Gln 205 | ccc Pro | ttt Phe | tac Tyr | atg Met | cac His 210 | atg Met | cct Pro | gca Ala | tcg Ser | 678 |
| act Thr 215 | cta Leu | | aag Lys | tgt Cys | cct Pro 220 | tct Ser | tgt Cys | gag Glu | aga Arg | atc Ile 225 | ggc Gly | ggc Gly | ggc | ggc Gly | 999 Gly 230 | 726 |
| | | gga Gly | gga Gly | gga Gly 235 | ggt Gly | ggc Gly | ggc Gly | agc Ser | 999 Gly 240 | gct Ala | acc Thr | gcg Ala | gtg Val | att Ile 245 | | 774 |
| gat Asj | gga Gly | agt Ser | acg Thr | Ala | aaa Lys | gga Gly | gct Ala | ttc Phe 255 | tct Ser | atc Ile | tcc Ser | tca Ser | aag Lys 260 | FIC | cac His | 822 |
| tto Pho | tto Phe | aac Asn 265 | Pro | ttt Phe | act Thr | aac Asn | cca Pro 270 | ser | gca Ala | gct Ala | tgt Cys | tga | ata | gtta | att | 871 |
| cg | ttaa | | | actt | aa a | atat | taat | t tt | cttt | tttt | ttt | tggg | tgg | catt | tt | 927 |
| _ | | | | | | | | | | | | | | | | |
| <2 <2 | 10> 11> 12> 13> | 54 274 PRT Aral | oidop | sis | thal | iana | ı | | | | | | | | | |
| <4 | 00> | 54 | | | | | | | | | | | | | | |
| Me 1 | t Gl | y Phe | e Ası | Asp 5 | Thr | Сув | Asn | Thr | Gly 10 | Leu | ı Val | . Lev | Gly | / Let 15 | ı Gly | |
| Pr | o Se | r Pro | 20 | e Sei | - Asn | Aer | туг | Asn 25 | ser | Thi | : Ile | e Arg | Glr 30 | n Sei | c Ser | |
| Va | 1 Ту | r Ly 35 | s Le | u Glı | ı Pro | Sei | Let 40 | ı Thr | . Let | ı Cys | s Lev | 1 Se1 45 | c Gly | у Авј | Pro | |
| Se | r Va 50 | | r Va | l Va | l Thi | : Gly 55 | y Ala | a Asp | Gl: | ı Leı | u Cys 60 | s Arg | g Gl | n Th | r Ser | |
| Se 65 | | s Se | r Gl | y Va | 1 Sei 70 | : Se | r Ph | e Sei | r Sei | r Gl ₃ | y Arg | g Vai | l Va | l Ly | s Arg 80 | |
| G! | lu Ar | g As | p Gl | y Gl 85 | y Gli | ı Gl | u Se | r Pro | o Glv 90 | u Gl | u Gl | u Gl | u Me | t Th 95 | r Glu | |
| A | rg Va | 1 11 | e Se | | р Ту | r Hi | s Gl | u As 10 | p Gl | u Gl | u Gl | y Il | e Se 11 | r Al 0 | a Arg | |

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MBI16 Sequence Listing.ST25

Lys Lys Leu Arg Leu Thr Lys Gln Gln Ser Ala Leu Leu Glu Glu Ser 115 120 125

Phe Lys Asp His Ser Thr Leu Asn Pro Lys Gln Lys Gln Val Leu Ala 130 135 140

Arg Gln Leu Asn Leu Arg Pro Arg Gln Val Glu Val Trp Phe Gln Asn 145 150 150 160

Arg Arg Ala Arg Thr Lys Leu Lys Gln Thr Glu Val Asp Cys Glu Phe 165 170 175

Leu Lys Lys Cys Cys Glu Thr Leu Ala Asp Glu Asn Ile Arg Leu Gln 180 185 190

Lys Glu Ile Gln Glu Leu Lys Thr Leu Lys Leu Thr Gln Pro Phe Tyr 195 200 205

Met His Met Pro Ala Ser Thr Leu Thr Lys Cys Pro Ser Cys Glu Arg 210 215 220

Ile Gly Gly Gly Gly Gly Gly Asn Gly Gly Gly Gly Gly Gly Ser Gly 225 230 235

Ala Thr Ala Val Ile Val Asp Gly Ser Thr Ala Lys Gly Ala Phe Ser 245 250 255

Ile Ser Ser Lys Pro His Phe Phe Asn Pro Phe Thr Asn Pro Ser Ala 260 265 270

Ala Cys

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| IPC(7) 11/00 | SSIFICATION OF SUBJECT MATTER : C12N 5/04, 5/10, 15/00, 15/09, 15/63, 15/70, | | H 21/02, 21/0 | 4; A01H 1/00, 9/00, | | | | |
| US CL | <u>: 435/320.1, 419; 468; 536/23.1; 800/ 278, 295</u> | | | | | | | |
| B. FIEL | DS SEARCHED | , | | | | | | |
| | cumentation searched (classification system followed 35/320.1, 419, 468; 536/23.1; 800/ 278, 295 | by classification symbols) | | | | | | |
| Documentati | on searched other than minimum documentation to th | e extent that such documen | as are included | in the fields searched | | | | |
| | ta base consulted during the international search (nai ontinuation Sheet | ne of data base and, where | practicable, se | earch terms used) | | | | |
| | UMENTS CONSIDERED TO BE RELEVANT | | | | | | | |
| Category * | Citation of document, with indication, where a | | | Relevant to claim No. | | | | |
| P,X | Database GenEmbl on STIC, USPTO, (Arlington, | | | 4-6 | | | | |
| P,Y | AC002388, LIN et al. 'Sequence analysis of chrom thaliana,' abstract, Nature, 1999, Vol. 402, 761-76 | | dobsis | 1-3, 7-13, 25-27 | | | | |
| P,X | Database EST on STIC, USPTO, (Arlington, VA, | USA), GenBank Accession | | 4-6 | | | | |
| P,Y | ASAMIZU et al. 'A large scale analysis of cDNA i 12,028 non-redundant expressed sequence tags from libraries,' abstract, DNA Research, 2000, Vol. 7, | n normalized and size-selec | | 1-3, 7-13, 25-27 | | | | |
| x | Database EST on STIC, USPTO, (Arlington, VA, | USA), Genbank Accession | A1997809, | 4-6 | | | | |
| Υ . | CHEN et al. unpublished, abstract, 08 September 1999. | | | | | | | |
| X | Database EST on STIC, USPTO, (Arlington, VA, NEWMAN et al. 'Genes galore: a summary of met | hods for accessing results f | rom large- | 4-6 | | | | |
| Y | scale partial sequencing of anonymous Arabidopsis Physiology, 1994, Vol. 106, 1241-1255. | cDNA clones, abstract, Pl | lant | 1-3, 7-13, 25-27 | | | | |
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| Further | documents are listed in the continuation of Box C. | See patent famil | y annex. | | | | | |
| • s | pecial categories of cited documents: | | | national filing date or priority | | | | |
| | defining the general state of the art which is not considered to be lar relevance | principle or theory t | inderlying the inves | | | | | |
| "E" earlier ap | plication or patent published on or after the international filing date | | cannot be consider | laimed invention cannot be ed to involve an inventive step | | | | |
| establish t | | | | | | | | |
| "O" document | | | | | | | | |
| | published prior to the international filing date but later than the ate claimed | "&" document member of | of the same patent f | amily | | | | |
| | ctual completion of the international search | Date of mailing of the int | emational sear | ch report | | | | |
| | 2001 (13.02.2001) siling address of the ISA/US | Authorized officer | | _ | | | | |
| | unissioner of Patents and Trademarks | / i | 000/ | ellino Joz | | | | |
| Box | PCT hington, D.C. 20231 | Cynthia Collins | | muno tak | | | | |
| _ | o. (703)305-3230 | Telephone No. (703) 603 | 5-1210 | | | | | |

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| Category* Citation of document, with indication, where appropriate, of the relevant passages Database EST on STIC, USPTO, (Arlington, VA, USA), GenBake Accession ANSHR3, NEWMAN et al. Genes galories: a summary of methods for accessing results from large-scale parial sequencing of anouymous Arabidopsis cDNA clones, abstract, Plant Physiology, 1994, Vol. 106, 1241-1255. Z Database PIR 66 on STIC, USPTO, (Arlington, VA, USA), Accession T00409, ROUNSLEY et al. unpublished, abstract, 01 February 1999. Z Database STREMBL_15 on STIC, USPTO, (Arlington, VA, USA), Accession 022167, ROUNSLEY et al. unpublished, abstract, 01 January 1998. T.E. RIECHMANN et al. Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. Science. 15 December 2000, Vol. 290, pages 2105-210. P.A. SUNG et al. Developmentally regulated expression of two MADS-box genes, MdMADS3 and MdMADS4, in the morphogenesis of flower bods and fruits in apple. Fluaria. March 2000, Vol. 210, pages 315-328. P.Y. RIECHMANN et al. A genomic perspective on plant transcription factors. Current Opinion in Plant Biology. October 2000, Vol. 32, pages 423-438, especially pages 427-428. V. S. SR2,009 A (THOMASHOW et al.) 06 April 1999, column 14, lines 1-46. RATCLIFFE et al. Separation of shoot and floral Identity in Arabidopsis. Development. March 1999, Vol. 126, pages 1190-1120. A. SUNG et al. Characterization of MdMADS2, a member of the SQUAMOSA subfamily of genes, in apple. Plum Physiology, August 1999, Vol. 120, pages 909-978. A. RIECHMANN et al. Development of their DNA-binding specificity. Molecular Biology of the Cell. July 1997, Vol., pages 1243-1259. A. HEARD et al. Evolutionary diversity of symbiotically induced nochide MADS box genes: characterization of ambCS, a member of a novel subfamily, Molecular Plant-Microbe lateracticus. July 1997, Vol. 10, No. 5, pages 105-110. A. RIECHMANN et al. DNA-binding properties of Arabidopsis MADS domain homeotic proteins API, API, Pages 3107-3114. A. RIECHMANN et al. DNA-b | C (Continu | nation) DOCUMENTS CONSIDERED TO BE RELEVANT | |
|--|------------|--|-------------------------|
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| Database PIR 66 on STIC, USPTO, (Arlington, VA, USA), Accession T00409, ROUNSLEY et al. unpublished, abstract, 01 February 1999. Database SPIR 66 on STIC, USPTO, (Arlington, VA, USA), Accession T00409, ROUNSLEY et al. unpublished, abstract, 01 February 1999. The property of the p | | Develore EST on STIC USDIC) (Arlington, VA. USA), GenBank Accession AA598183, NEWMAN | |
| unpublished, abstract, 01 February 1999. To Database SPTREMBL_15 on STIC, USPTO, (Arlington, VA, USA), Accession 022167, ROUNSLEY et al. unpublished, abstract, 01 January 1998. RIECHMANN et al. Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. Science. 15 December 2000, Vol. 290, pages 2105-2110. SUNG et al. Developmentally regulated expression of two MADS-box genes, MdMADS3 and MdMADS4, in the morphogenesis of flower bods and fruits in apple. Planta. March 2000, Vol. 210, pages 1919-232. P.Y | | et al. 'Genes galore: a summary of methods for accessing results from targe-scale partial sequencing of anonymous Arabidopsis cDNA clones,' abstract, Plant Physiology, 1994, Vol. 106, 1241-1255. | 1-3, 7-13, 25-27 |
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International application No.

PCT/US00/31458

| Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet) |
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| This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons: |
| 1. Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely: |
| Claim Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically: |
| 3. Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a). |
| Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet) |
| This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet |
| 1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims. |
| 2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite |
| payment of any additional fee. 3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: |
| 4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-13, 25-27 SEQ ID NOS: 1 and 2 |
| Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees. |

nai application No.

PCT/US00/31458

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I-XXVII, claim(s) 1-13 and 25-27, drawn to transgenic plants with modified environmental stress tolerance, polynucleotides and vectors for producing said transgenic plants, and methods of making said transgenic plants. Applicant must elect one pair of sequences (one nucleotide sequence and its corresponding amino acid translation) per Group to be examined, i.c. SEQ ID NOS: 1 and 2 in Group I, SEQ ID NOS: 3 and 4 in Group II, SEQ ID NOS: 5 and 6 in Group III, etc.

Group XXVIII, claim(s) 15-17, drawn to a method of identifying a factor that is modulated by or interacts with a polypeptide.

Group XXIX, claim(s) 18, drawn to a method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest.

Group XXX, claim(s) 19 and 20, drawn to an integrated system, computer, or computer readable medium.

Group XXXI, claim(s) 21-23, drawn to a method of identifying a polynucleotide sequence.

The inventions listed as Groups I-XXXI do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I-XXVII are drawn to transgenic plants and methods of producing said plants with nucleic acid sequences. The methods of Groups I-XXVII differ from each other in that they are directed to plant transformation methods and transgenic plants with structurally and functionally distinct nucleic acid sequences which encode structurally and functionally different amino acid sequences. In addition, Groups XXVIII, XXIX, and XXXI are different methods from any of Groups I-XXVII in that they have different method steps and different end products, and Group XXX requires a computer system. Thus, there is no single special technical feature which links the inventions of Groups I-XXXI under PCT Rule 13.2.

Continuation of B. FIELDS SEARCHED Item 3: STN (agricola, biosis, biotechno, biotechds, biotechabs, caba, caplus, embase, medline, uspatfull, wpids, pctfull, europatfull, japio) SEARCH TERMS: inventor names, plant transcription factor, stress tolerance; STIC sequence search for SEQ ID NOS: 1 and 2